Applications of High Voltage Power Supplies in the Purification of Water

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ABSTRACT

High voltage treatment technology has been developed in this thesis and had initially shown promise in its effectiveness in reducing microorganisms found in water supplies. Initial testing found that the high voltage could destroy over 99.9% of the bacteria *S. marcescens* (a 3-log reduction). Cited literature on the effects of high voltage pulsed electric fields (PEFs) on various microorganisms have shown that high destruction rates of up to 9-log can be achieved. Thus by increasing the electric field strength or exposure time, or by improving the design of the electrode flow chamber, better results should be achieved using high voltage on water. However, contrary to this, upon further design improvements the 99.9% destruction threshold was rarely increased. The initial slow flow device of one litre-per-minute (1 LPM) was scaled up to flows of 10 LPM and 33 LPM. However, these faster flow devices were even less effective in the destruction of bacteria, destroying only 99% of *S. marcescens* (2-log reduction). No physical or technical design parameters could account for this low performance. One possible reason for these low results was in the preparation of the bacteria themselves. It was discovered that the growth stage of bacteria prepared for experiments had a large effect on the results. Bacteria harvested in the early growth stage could be nearly all destroyed by the high voltage (>4-log reduction), whereas those harvested in the late stationary stage were much more resistant (<0.5-log reduction). Bacteria naturally occurring in water supplies will mostly be in a non-metabolising state. This implies that they will be more resistant to high voltage exposure than bacteria grown in a laboratory under standard testing procedures. Thus standard testing procedures for this device do not give accurate results.

Further research into the mechanism behind the bacterial resistance is required to improve the performance of high voltage devices. A combination of different technologies may also prove effective in overcoming the resistance mechanism. These improvements are required before high voltage treatment can be properly developed and commercially exploited.

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GLOSSARY

bacteria - organisms with prokaryotic cells.

cell viability - the measure of the ability of a cell to procreate or reproduce. Non-viable cells are unable to reproduce.

Coulter counter - a device used in the counting and sizing of microscopic particles in conducting fluids. This device was first developed by Coulter (1953). The transducer consists of a small orifice through which the suspension is pumped, producing a change in electrical resistance as each particle traverses the orifice.

cytoplasm - all internal constituents of a cell excluding the cell membrane and the genetic material.

cytoplasmic membrane - or *cell membrane*, a phospholipid bilayer that surrounds the constituents of all cells.

disinfection - the destruction, inactivation, or removal of microorganisms likely to cause infection or produce other undesirable effects.

E10 - name for the 10 LPM high voltage treatment device.

exposure time - the length of time a suspended particle lies directly between the electrodes during treatment.

integral protein - a membrane protein which interacts with the inner hydrophobic regions of the membrane.

lipopolysaccharides - molecules consisting of covalently linked lipids and poly-saccharides; a component of Gram-negative bacterial cell walls.

LPM - Litre per minute. A measure of flow rate.

nucleus - membrane-bound region within a cell where the genome or set of genes is contained. This is found in all eukaryotic cells but is not found in prokaryotic cells such as bacteria.

organelle - a membrane-bound structure that forms part of a microorganism and that performs a specialised function.

PEF treatment - Pulsed Electric Field treatment. The application of high magnitude 'pulses' of electric field to an electrode chamber usually containing some liquid or semi-solid food.

peptidoglycan layer - the rigid component of the cell wall in most bacteria.

phospholipid - a lipid compound that is an ester of phosphoric acid and also contains one or two molecules of fatty acid, an alcohol, and sometimes a nitrogenous base.

porins - proteins found in the outer membranes of gram-negative cells in groups of three. They form transmembrane channels through which small molecules can diffuse.

prokaryote - single celled organisms that do not have a defined nucleus.

protozoa - diverse eukaryotic, typically unicellular, nonphotosynthetic microorganisms generally lacking a rigid cell wall.

SMPS - Switch-Mode Power Supply. A high frequency square-wave source of high voltage.

UV - Ultraviolet. UV waveforms are used in water disinfection systems to destroy bacteria and viruses by disrupting the DNA.

vegetative cells - cells that are engaged in nutrition and growth; they do not act as specialised reproductive or dormant forms.

virus - a non-cellular and non-living entity that can invade cells and use the cell machinery to make copies of itself.

water treatment - the process necessary to bring water to the required quality for a specific application. This may include the addition of chemicals to disinfect against bacterial pathogens.

water purification - the process of removing impurities from the water so the remaining fluid is pure.

Wateriser - name for a high voltage treatment device that operates on a flow of 1 LPM. This device uses a 50 Hz high-voltage supply.

PREFACE

The development of high voltage technology as a method of water disinfection has generated considerable commercial and industrial interest. It indeed has many possible applications worldwide as the pollution and availability of potable water supplies becomes an increasingly important issue.

The original development of the Wateriser was completed in 1997 as part of an ME thesis. A paper detailing the Wateriser process was presented at both the IPENZ 3rd conference of Postgraduate Students in Engineering and Technology 1996 and the IPENZ National Conference 1997. There was considerable interest in the device. The technology was subsequently patented in a number of different countries (US Patent No. 5,851,375). In April 1996 the Wateriser was displayed at the Royal Easter Show (Auckland, New Zealand) where it won a 'highly commended' award in the inventors section. The Wateriser also won the 'Best New Idea' category at the national ECNZ Rutherford Awards, 1996.

Following on from 1997, work was undertaken both on the scaling up and further development of the device, as well as research toward a better scientific understanding of the process itself. This work is documented in this thesis.

Chapters 1-3 contain a general review and introduction to this topic. The aim of these chapters is to introduce the reader to the basics of water treatment and the effects of high magnitude electric fields on cells. Chapter 3 contains a summary of the pulsed electric field (PEF) treatment method of liquid foods. This method is currently under much research in laboratories throughout the world. The technology is similar to the Wateriser process and needs to be well understood as background material to the thesis. Chapter 4 contains a brief summary of the development of the Wateriser and is taken mainly from the ME thesis.

The further research and development that is a part of this doctorate is documented in Chapters 5-16.

Commercialisation of the Wateriser was undertaken during 1997-1998. During this time, 6 months was spent in Kuala Lumpur, Malaysia, transferring the technology to a manufacturing company, Abric Berhad. A summary of the resulting plan for commercialisation is given in Appendix A. The finished commercialised device was rigorously tested to compare with the initial demonstration model. This testing is documented in Chapter 5.

The Wateriser was also tested on an important water-bourne pathogen, *Giardia intestinalis*. The results from this testing are given in Chapter 6.

Chapters 7-11 document work done in the scaling up of the technology to industrial flow rates. Chapter 7 looks at the design changes necessary to achieve this for a 10 LPM

flow rate. Chapter 8 documents some problems associated with the scaling up of this technology, and lists design modifications to overcome these. Chapter 9 details a microcontroller design to control the added functions of the large flow rate devices and give more flexibility. Chapter 10 details a 33 LPM device. Chapter 11 looks at the relative performance of the 10 & 33 LPM devices when compared to the Wateriser. The large flow rate devices are not as effective in lysing bacteria. Different reasons are explored to account for this.

A high voltage SMPS is applied to water treatment. The relative performance of this is examined in Chapter 12. The SMPS has a number of significant advantages over 50 Hz treatment, however does not work as effectively.

Chapter 13 looks into the treatment of recirculating systems such as air conditioning. Careful design of such systems may enable operating and energy savings to be made. A point-of-use treatment method may be operated at sub-optimum levels, cost less and be more effective in keeping bacteria levels down.

The climax of the thesis is given in Chapter 14. This work shows that the effectiveness of high voltage treatment systems is highly dependent on the physiological state of the bacteria themselves. Bacteria in an active state of growth may be more readily destroyed than those in a non-metabolising state. This factor is shown to be the most important parameter investigated that dictates the effectiveness of high voltage treatment.

There is a discussion on future work in Chapter 15. This chapter gives many ideas for further research and raises more questions that need to be explored before this technology can perhaps become commercially useful.

Paper and conference publications arising from this thesis

JOHNSTONE, P.T., BODGER, P.S., AND KLENA, J.D.: "Effects of cell growth phase on the inactivation of bacteria using high voltage", *Applied and Environmental Microbiology, under review.*

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JOHNSTONE, P.T. AND BODGER, P.S.: "Applications of a high voltage SMPS in water disinfection", *IEE Proc. Science, Measurement and Technology*, Vol. 148, No. 2, March 2001.

BODGER, P.S., LIEW, M.C. AND JOHNSTONE, P.T.: "A comparison of conventional and reverse transformer design", *AUPEC2000*, Brisbane, Australia, 24-27 September, 2000, pp. 80-85.

JOHNSTONE, P.T. AND BODGER, P.S.: "Disinfection of deionised water using AC high voltage", *IEE Proc. Science, Measurement and Technology*, Vol. 147, No. 3, May 2000, pp. 141-144.

BODGER, P.S., JOHNSTONE, P.T., AND JACQUIERY, A.G.: "Apparatus for Disinfecting Fluids", US Patent 5,851,375, 1998.

JOHNSTONE, P.T. AND BODGER, P.S.: "High voltage disinfection of liquids", *IPENZ Trans.*, v24, n1, EMCh, November 1997, pp. 30-35.

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Chapter 1

WATER TREATMENT SYSTEMS

1.1 Water Quality

Water is an essential element in the maintenance and composition of all forms of life. Most living organisms can survive only for short periods of time without water. There is often a direct relationship between the abundance of good quality water, population density and quality of life. The effects of a lack of quality water on human life can be seen most vividly in the third-world or developing countries, where sixty percent of all persons live without an adequate supply of drinking water [Dangerfield 1983]. Since water is usually remote as well as polluted, tens of millions of women and children spend eight or more hours daily fetching polluted water. The cost in human suffering is enormous. Sickness and infection are spread through polluted water supplies and poor sanitation. Unsafe water causes high child mortality rates and poor health for those that do survive. A report published by the United Nations in 1980 quoted that more than 25,000 people die every day due to inadequate water supply and sanitation [Dangerfield 1983]. At the time of the report the situation was rapidly deteriorating.

Human beings intercept the natural water cycle in order to take water for their purposes, and after using the water, they return it to the cycle. During this usage the water often becomes polluted. Pollution can also occur in other stages of the natural water cycle. In developed countries, water treatment and distribution follow processes such as that described in Figure 1.1. Water treatment is required in the process to reduce water pollution and make the water potable.

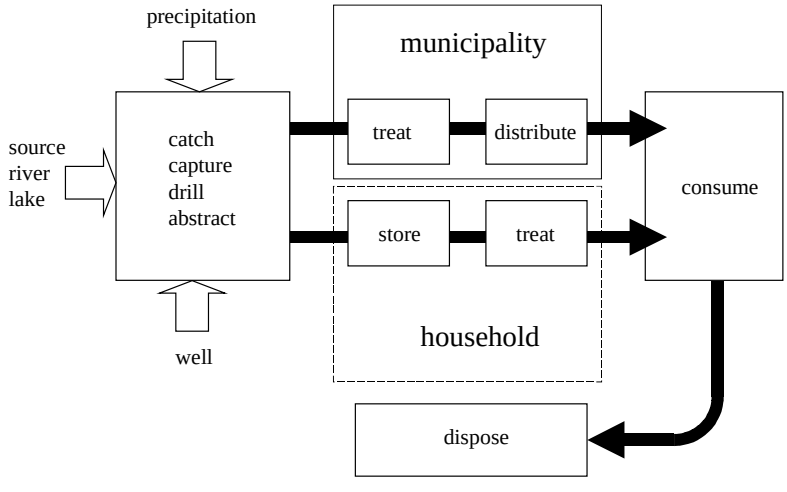


Figure 1.1 Drinking water flow cycle [Heber 1985].

The most important objective of water treatment is to produce a water supply that is biologically and chemically safe for human consumption. Water must be free from organisms that are capable of causing disease and from chemical substances that could produce adverse physiological effects.

Generally the water quality requirements for domestic use will apply for most industrial users. In some cases, such as the manufacture of printed-circuit boards and in pharmaceutical industries, even higher quality requirements may have to be met. Water contamination or pollution may be divided into three main categories: microbiological, inorganic and organic contamination.

1.1.1 Microbiological Contamination

Residents of New Zealand have been very fortunate in that there has been ample supplies of fresh and pure water from mountains, rivers and underground aquifers. These sources of quality water, however, have been rapidly deteriorating over the past decade as the mobility of the population increases. As immigrants and world travellers enter the country they can bring with them bacteria and diseases from other countries. Pathogens such as *Giardia intestinalis*, *Cryptosporidium parvuum* and *Campylobacter jejuni* are now prevalent in many of the country's rivers, lakes and water supplies.

1.1.1.1 *Giardia intestinalis*

Giardia intestinalis is a parasitic protozoan which causes disease in human hosts. *Giardia* exists in two different forms; the *cyst* form outside of the host, and the *trophozoite* form inside the host. In cyst form the parasite is dormant and a thick cell wall encases the organism. More details of this structure is given in Section 2.2. Cysts can survive outside the host for over two months. When they enter the host they lodge in the small intestine where they hatch into two trophozoites which then rapidly multiply.

The symptoms of the disease giardiasis are explosive, foul smelling diarrhoea, abdominal cramps, flatulence, dehydration, nausea, and weight loss. Symptoms can last for up to four or five weeks. Infected hosts pass *Giardia* cysts in their faeces. Cysts can survive dormant for two months before a new host becomes infected.

1.1.2 Inorganic Compounds

Water is a highly effective solvent because it has a high dielectric constant and the water molecule has the ability to share or donate electrons. For this reason, all natural waters contain dissolved ionic constituents. The most common of the ions are listed in Table 1.1 [Tchobanoglous and Schroeder 1985].

CATIONS	ANIONS
Calcium (Ca^{+2})	Bicarbonate (HCO_3^-)
Magnesium (Mg^{+2})	Sulfate (SO_4^{-2})
Sodium (Na^+)	Chloride (Cl^-)
Potassium (K^+)	Nitrate (NO_3^-)

Table 1.1 Major ionic species found in water

The amount and type of the ionic species found in water supplies is mainly determined by the contact of the water with various mineral deposits. Thus rainwater often has less ionic content than surface or groundwater supplies. As ions act as charge carriers when an electric current is passed through the water, the ionic content directly determines the *conductivity* of the water. This has a direct bearing on the design and performance of an electrical water disinfection unit, as is discussed in later chapters.

The amount of multivalent cations, particularly magnesium and calcium, determine the **hardness** of the water. Water hardness does not effect drinking water quality, although some heavy metals such as arsenic, lead and cadmium discharged in waste-water can present health concerns. Water hardness causes problems with scaling in pipes and industrial systems, and reacts with soap to form a difficult-to-remove scum.

1.1.3 Organic Compounds

The amount of organic matter present in most natural waters is low. Typically, the source of organic matter is from decaying weeds, leaves and trees. These are *natural* organic compounds, and form humic acid. Other contaminants that cause concern are *synthesised organic compounds*, such as detergents, pesticides, herbicides and solvents. There have been more than 100,000 organic compounds synthesised since 1940 [Tchobanoglaus and Schroeder 1985]. These can contaminate natural waters as a result of agricultural run-off or industrial discharge and can cause major health problems if left unchecked.

1.2 Water Disinfection Methods

Disinfection is the term applied to the selective destruction of disease-causing organisms. Generally the aim is to reduce the numbers of viable organisms in the water or liquid to acceptable and less harmful levels. The complete destruction of all organisms is termed sterilisation. This is best achieved by the application of high temperatures and high pressures in an autoclave.

1.2.1 Chlorination

Perhaps the most widely used method of water disinfection throughout developed countries is the application of chlorine in the form of Cl_2 gas, chlorine dioxide (ClO_2), sodium hypochlorite (NaOCl) or calcium hypochlorite ($Ca(OCl)_2$). When chlorine is added to water, two reactions occur - hydrolysis and ionisation. In the hydrolysis reaction, hypochlorite (HOCl) is formed (Equation 1.1). In the ionisation reaction, the chlorite ion (OCl^-) is formed (Equation 1.2).

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^- \tag{1.1}$$

$$HOCl \rightleftharpoons H^+ + OCl^- \tag{1.2}$$

Hypochlorite is a more effective disinfectant than the chlorite ion, and is involved in the destruction of viruses, bacteria and protozoans. The effectiveness of this disinfection process depends on a number of factors, including concentration of

chlorine, contact time, type and concentration of organisms. For example, at the same HOCl concentration, deactivation of the *Coxsackie virus* requires about 500 times the contact time necessary for deactivation of the *adenovirus* [Berg 1964]. In general, bacteria are much easier to destroy using chlorine than viruses [Tchobanoglous and Schroeder 1985]. Many of the protozoa of concern in water supplies, such as *Giardia lamblia*, exist in encysted states that are more resistant to chlorine. Free residual chlorine is a measure of the available chlorine in solution, defined as the sum of [HOCl] and [OCl^-]. The higher the amount of free residual chlorine in solution, the more destructive the effect on organisms.

A major disadvantage in using chlorine in the disinfection of water is that it can result in the production of carcinogenic compounds such as trihalomethanes and chloroform [Tchobanoglous and Schroeder 1985]. Correct procedures must be observed to minimise health risks in the handling of chlorine. Another important disadvantage of chlorine disinfection associated with drinking water is that it alters the taste and smell of the water.

1.2.2 Ultraviolet Light

Ultraviolet (UV) radiation covers the wavelength range from 100-400 nm. The wavelength range of UV which is germicidal is between 150-300 nm. Commercial UV disinfection units that are designed for water or other clear liquids are available. The basic design uses a suitable UV lamp surrounded by a quartz or teflon sleeve, through which the water passes. As the water passes in close proximity to the lamp the UV penetrates into the water and disinfection takes place. UV disinfection of water generally employs low-pressure mercury vapour lamps, which generate short-wave UV in the region of 253.7 nm. These lamps have a finite lifetime and generally require replacing after about 7500 hours of use. Occasionally the sleeving in contact with the water will require cleaning, especially when they are made of quartz. UV disinfection units are supplied in a range of sizes and are usually run in parallel to achieve high flow rates for large-scale applications.

Ultraviolet light is absorbed by many cellular materials, but most significantly by the nucleic acids (DNA and RNA). The UV radiation damages the DNA of organisms in the water and restricts their replication and growth. As with chlorine, the effectiveness of a UV disinfection unit depends on a number of factors, including radiation intensity, exposure time and turbidity of the water.

One of the major disadvantages of using UV light for the disinfection of organisms is that UV has very little ability to penetrate matter (even a thin layer of glass filters off a large percentage of the light). This means that UV becomes less effective in turbid water because the high number of suspended particles tend to absorb or disperse the UV radiation. In most commercial applications, the turbidity of the water to be treated must be below a certain threshold level before UV disinfection can be effectively utilised.

Another disadvantage of using UV disinfection technology is that UV is not normally effective on two of the main pathogenic organisms found in water that can cause sickness in humans, *Giadia lamblia* and *Crytosporidium parvum*. However, some recent research has indicated these organisms can be destroyed by using higher dosages of UV light [Campbell *et al*. 1995, Clancy *et al*. 1998, Karanis *et al*. 1992]. These findings have yet to be applied to most commercially available UV devices.

1.2.3 Ozone

Ozone is one of the most powerful oxidising agents readily available. It is an unstable, reactive and slightly soluble gas (O_3), which is generated by the electrical breakdown of air or oxygen. A typical corona type generator consists of a series of concentric cylindrical electrodes to which a moderately high voltage is applied (usually between 8 - 20 kV). Heat is generated in the process, and an electrode cooling system is required. Ozone produced by a corona generator must be applied immediately to the water as it has a half-life of between 2 and 18 minutes. In water, ozone may react directly with dissolved substances or organisms, or it may decompose to form secondary oxidants, such as •OH radicals, which then themselves immediately react with solutes. Ozone decomposition is a complex chain radical process in which there may be many intermediate steps. However, the overall stoichiometry is shown in Equation 1.3.

$$2O_3 \rightarrow 3O_2 \qquad (1.3)$$

Ozone is more effective than chlorine for the removal of protozoans *Giardia* and *Cryptosporidium*, viruses and certain forms of algae [Glaze 1987]. This is one reason ozone is being increasingly used for water disinfection.

Ozone disinfection technology is relatively expensive when compared to other methods. It requires a large capital cost to install, and involves considerable maintenance and monitoring. The high reactivity of ozone is both an advantage and a disadvantage. Its high reactivity means ozone readily attacks cells suspended in solution, and breaks down unwanted organic compounds into simpler constituents. However, the high reactivity and oxidation of ozone can generate various chemical compounds depending on the chemical composition of the water. The levels of ozone present in the water and some of the undesirable by-products must therefore be monitored.

1.2.4 Distillation

Boiling water is a form of disinfection. All vegetative cells in the water will be destroyed within minutes of continuous boiling. However, some bacterial spores can withstand this condition for many hours [Pelczar *et al*. 1986]. (For this reason the process of boiling water should be termed *disinfection* and *not sterilisation*).

In the distillation process, water is heated to its boiling point (100°C at normal pressure) and becomes vapour or steam. Impurities are thus separated from the water. The steam is then condensed back into liquid form and collected into a separate storage chamber. The impurities, including living organisms that survive the boiling process, remain in the residue, which is then discarded.

Distillation removes particulate matter, microorganisms and inorganic minerals. The process can also remove organic compounds by including a special vent for volatile gases. In many commercial distillation units, a carbon filter is also added to the process to remove any remaining organic compounds.

The distillation process is slow - commercial units are available that will provide 0.6 -1.5 litres per hour. Distillation is usually only used for small applications, such as personal drinking water. The process is expensive in terms of electrical power, requiring

between 500 and 1500 Watts. That is, the amount of energy required to distil one litre of water is about one kilo-watt hour.

1.2.5 Comparison of Available Technologies

Other commercial methods of water treatment are filtration and reverse osmosis. These methods however are not disinfection techniques, as they do not destroy organisms in the water, they simply filter them out. For this reason they are not mentioned in detail in this section.

	Impurity			
Water Treatment	***Particlulate Matter***	***Biological Organisms***	***Inorganic Compounds***	***Organic Compounds***
Wateriser	◗	●	●	◗
Chlorine	❍	●	❍	❍
Ultraviolet	❍	●	❍	❍
Ozone	❍	●	❍	◗
Distillation	●	●	●	◗
Carbon Filtration	●	◗	❍	●
Reverse Osmosis	●	●	❍	❍

❍ = little or no effect
◗ = partial reduction
● = complete or significant reduction

Table 1.2 Impurity removal for different water treatment technologies

A comparison between each method of water treatment and how they affect the quality of the water is given in Table 1.2. The high voltage disinfection "Wateriser" process, the topic of this thesis, is listed at the top of the table. This includes both the deionising column and the high voltage treatment. From this information it can be seen that by using a carbon filter in conjunction with a Wateriser unit results in water of very high quality. The Wateriser removes biological contaminants and inorganic compounds, and the carbon filter removes particulate matter and organic compounds.

Chapter 2

ELECTRIC FIELD EFFECTS ON BIOLOGICAL CELLS

2.1 Classes of Biological Cells

The two main classes of cells are prokaryotic and eukaryotic cells. Prokaryotic cells have a relatively simple structure and lack internal compartments and organelles. The genetic material (DNA) lies free within the cell. These types of cell are the most primitive. All bacteria are prokaryotes.

Eukaryotic cells are much more complex. They have an enclosed nucleus (the nucleus is surrounded by a nuclear membrane), and other organelles. Each subcellular component carries out specific functions. Eukaryotic cells are characteristic of animal cells or other 'higher' life forms.

2.2 Bacteria Cell Wall and Membrane Structure

The outer covering of a prokaryotic cell is composed of a cytoplasmic membrane underlying a strong and elastic murein or equivalent layer, and it may have an outer membrane and/or a protein array [Koch 1996]. This exoskeleton structure contains and retains the cytoplasm. The murein (or peptidoglycan) layer is required for shape determination and has important roles in cell division. It is a covalently linked fabric of carbohydrate chains cross-linked to flexible chains of a peptide polymer.

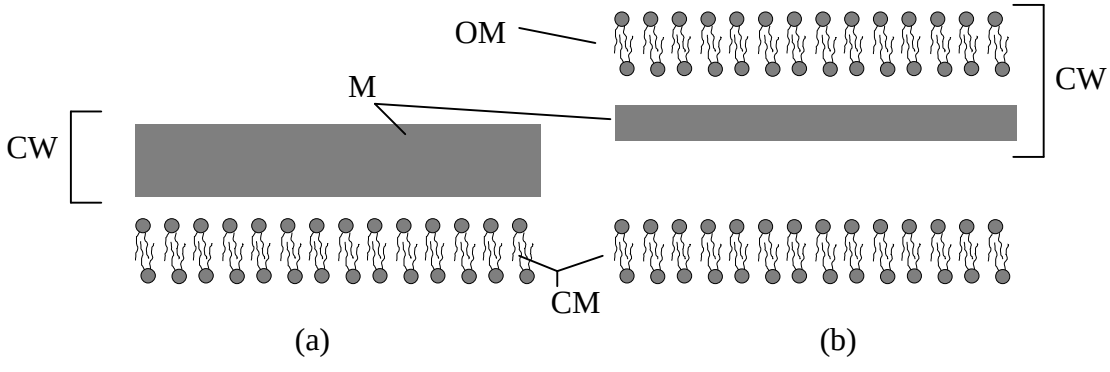


Figure 2.1 Basic cell wall structure of prokaryotes: (a) gram-positive (b) gram-negative. CM is the cytoplasmic membrane, M is the murein or peptidoglycan layer, OM the outer membrane and CW the cell wall [Atlas 1988].

Prokaryotic cell walls occur in two different configurations, depending upon their ability to readily take up certain types of dyes. These are known as gram-positive and

gram-negative cells. The structures of these are depicted in Figure 2.1. Gram-positive cell walls consist of a homogenous layer of peptidoglygans and polysaccharides that ranges from 10 to 80 nanometres in thickness [Curtis and Barnes 1989]. By contrast, the gram-negative cell wall consists of two layers: an inner peptidoglycan layer, usually 2 to 10 nanometres thick, and an outer membrane [Rogers *et al*. 1980]. The cell walls of both gram-positive and gram-negative cells enclose the cytoplasmic membrane.

The wall and membrane of *Giardia* cysts may be compared to that of gram-positive cells. A *Giardia* cyst has an outer wall that encloses a membrane. The outer wall is composed of a tightly packed layer of chitinous elements and is of the order of 300-500 nanometres thick [Kulda and Nohynkova 1978, Sheffield and Bjorvatn 1977]. This is much thicker than bacterial cells thus giving the cyst greater protection against the external environment.

The function of the cytoplasmic membrane is to maintain the integrity of the cell by regulating the passage of molecules into and out from the cell [Knox *et al*. 1994]. When this membrane is ruptured or loses its functionality, cell death may occur. The cytoplasmic membrane is composed largely of a bilayer of phospholipids, as shown in Figure 2.2. This molecule has a hydrophilic (affinity for water) head and hydrophobic (repulsion from water) tail of fatty acids. In addition to lipids, the membranes contain protein molecules. *Peripheral membrane proteins* are loosely associated with the membrane surface. *Integral proteins* interact with the inner hydrophobic regions of the membrane. Some of these proteins transverse the membrane and are called *transmembrane proteins*. Most of these proteins are involved with selective molecule transport across the membrane.

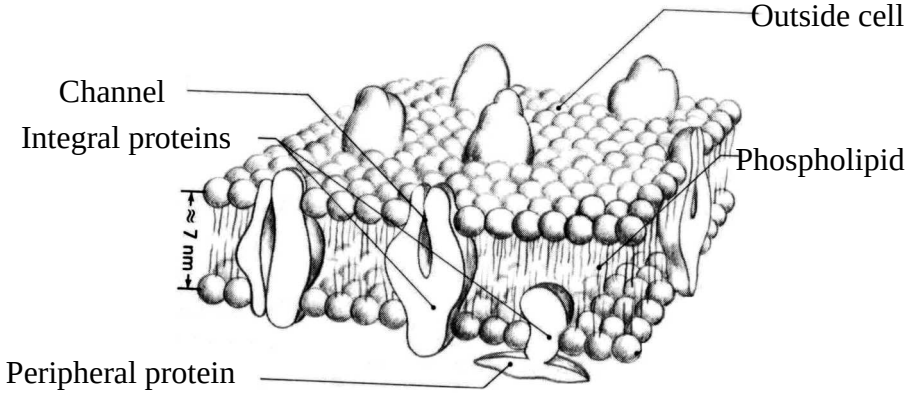


Figure 2.2 The fluid mosaic model of a cytoplasmic membrane structure [Atlas 1988].

The cytoplasmic membrane is called a fluid mosaic because lipid and protein molecules are free to move laterally across the membrane. The thickness of this membrane is about 7 nanometres. It is selectively permeable.

The outer membrane of a gram-negative bacteria is a lipid bilayer, similar to the cytoplasmic membrane, but it also contains lipopolysaccharides and porins [Atlas 1988]. Functionally, the outer membrane is a coarse molecular sieve that allows diffusion of small hydrophobic and hydrophilic molecules. This permeability to outside nutrients is partially caused by transmembrane proteins called porins. However, despite its permeability to small molecules, the outer membrane is less permeable than the cytoplasmic membrane to hydrophobic molecules. Therefore gram-negative bacteria are less sensitive to antibiotics having this property as the antibiotics are prevented from reaching their target inside the cell.

2.3 Electrical Equivalent Circuit of a Biological Cell

A simplified electrical equivalent circuit for a biological cell in suspension is shown in Figure 2.3 [Schoenbach *et al*. 1997a]. This circuit can be applied to basic prokaryotic cells. It does not take into account the effects of structures inside of cells, such as the nucleus and other organelles.

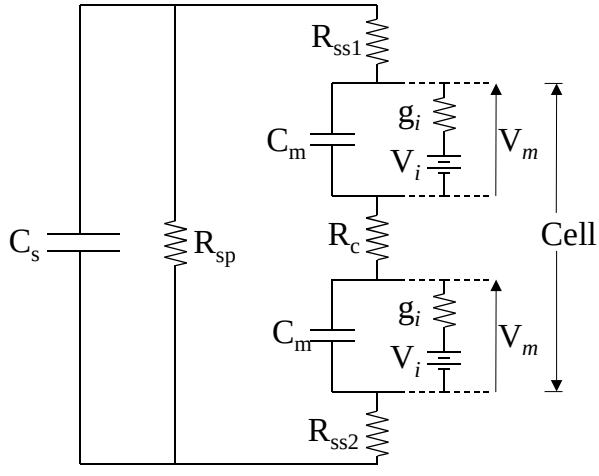


Figure 2.3 Electrical equivalent circuit of a cell in suspension.

The suspension is modelled by a resistance and capacitance. C_s is the capacitance of the suspension, R_{sp} is the parallel resistance of the suspension, R_{ss1} and R_{ss2} are the resistances of the suspension in series with the cell. For electrical exposure times much longer than the dielectric relaxation time of the suspension, the suspension capacitance can be neglected. The dielectric relaxation time for the suspension may be calculated by

$$\tau_s = \varepsilon_0 \varepsilon_r / \kappa \qquad (2.1)$$

where ε_0 is the permittivity of free space = 8.85 x 10^{-12} $C^2.N^{-1}.m^{-2}$, ε_r is the relative dielectric constant of the suspension, and κ is the conductivity. This thesis examines cells suspended in water. Water has a relative dielectric constant of ε_r = 81. For deionised water with conductivity, κ = 1 μS/cm, the dielectric relaxation time is τ_s =7.2 μs. For water with conductivity 100 μS/cm, τ_s decreases to 72 ns.

The cell is described by the capacitance of the cell membrane, C_m, in series with the resistance of the cell interior, R_c. A typical capacitance per unit area for cell membranes is 1 μF/cm^2 [Zimmermann 1986]. The cell interior has a resistivity, ρ_c, on the order of 100 Ω.cm [Cole 1937]. The integral proteins embedded into the membrane can act as voltage-gated channels. These channels provide a pathway for the flux of ions. This effect can be modelled by voltage-dependent conductances g_i (i = 1,2,...) in series with driving voltage sources V_i for each ion species [Hodgkin and Huxley 1952].

An externally applied electric field, E, will induce a voltage potential, V_m, across the cell membrane according to the formula

$$V_m = Efa \qquad (2.2)$$

where *f* is a form factor dependent on the shape of the cell [Sale and Hamilton 1968] and *a* is the cell radius.

Increasing the membrane voltage potential is not an instantaneous effect. A finite amount of time is required to charge up the membrane capacitance. The time constant for cell membrane charging is given by:

$$\tau_c = (\frac{\rho_s}{2} + \rho_c)ca \tag{2.3}$$

where ρ_c is the resistivity of the cell interior (~100 Ω.cm), ρ_s is the resistivity of the suspension, and *c* is the capacitance of the cell membrane per unit area (~1 μF/cm^2). For water with conductivity κ = 1 μS/cm, ρ_s = 1 MΩ.cm, and the membrane time constant is 25 μs. For water with conductivity 100 μS/cm, the time constant is 0.25 μs. These values are higher than those for the dielectric relaxation time of the water (7.2 and 0.072 μs respectively) and so determine the minimum effective exposure time of the applied electric field.

2.3.1 Spherical cells

The form factor in Equation 2.2 for spherical cells is *f* = 1.5. The membrane potential of a spherical cell suspended in solution is usually calculated using [Zimmermann 1986]:

$$V_c = 1.5E_c a\cos\alpha \pm V_m \tag{2.4}$$

where E_c is the critical external electric field, V_c is the critical membrane (breakdown) potential, V_m is the resting transmembrane potential, and α is the angle between the membrane site and the field direction.

The effect of this can be seen in Figure 2.4. The electropermeabilisation area induced by the electric field on the membrane is shown by the dotted lines. This area is larger on the side of the cell facing the positive electrode, due to the addition of the resting transmembrane potential to that induced. Conversely, the area on the cell side facing the negative electrode is smaller due to the subtracting effect.

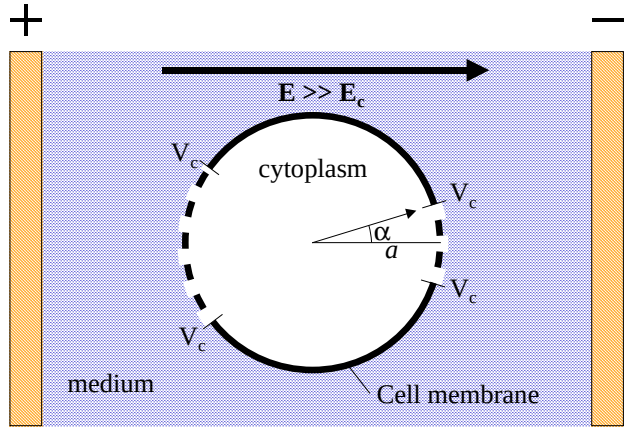


Figure 2.4 Spherical cell in suspension showing the areas of electropermeabilisation (dotted lines) due to an external electric field.

2.3.2 Rod shaped cells

The spherical cell model is the most studied because of its simplicity. However, most bacterial cells are non-spherical. Many bacteria types exist in the form of rods. This includes the bacteria *Serratia marcescens* and *Escherichia coli*, which are studied in this thesis.

Rod shaped cells can be modelled by a long cylinder with a hemisphere at each end. In this model the form factor can be given by

$$f = l/(l - d/3) \tag{2.5}$$

where l is the length of the rod and d the diameter [Zimmermann *et al*. 1974]. Substitution of the form factor f in Equation 2.2 allows an approximate calculation of the induced potential for rod shaped bacteria. This calculation corresponds to bacteria aligned with their long axis parallel to the electric field. In a real situation the bacteria orientation would be random.

Table 2.1 gives a list of physical parameters for a range of bacteria. The critical membrane potential necessary for the inactivation of most bacteria in the stationary phase is about 1 V. For *E. coli* in the early logarithmic stage of growth (4h incubation) the critical membrane potential is much lower (0.26 V). The cell diameter and length of *E. coli* in the early logarithmic stage of growth is much larger than *E. coli* in the stationary growth stage. The cell length after 4 hours incubation is more than three times that after 30 hours incubation. This size difference may have an effect on the susceptibility of *E. coli* to electric fields. The effects of growth phase on the effectiveness of high voltage treatment for these bacteria are explored in Chapter 14.

Bacteria	d (μm)	l (μm)	V (μm^3)	f	V_m (V)
E. coli (4 h)	1.15	6.9	7.2	1.06	0.26
E.coli (30 h)	0.88	2.2	1.4	1.15	1.06
K. pseudomonia	0.83	3.2	1.7	1.09	1.26
P. aeruginosa	0.73	3.9	1.6	1.07	1.25
S. aureus	1.03	-	0.6	1.50	1.00
L. monocytogenes	0.76	1.7	0.8	1.17	0.99

Table 2.1 Cell size and induced membrane potential of studied bacteria: d = mean diameter, l = mean length, V = mean volume, f = shape factor, V_m = membrane potential induced by an external electric field E_c under the assumption of a parallel long particle axis and field vector [Hulsheger *et al*. 1983].

Zimmermann *et al*. (1974) measured the size of *E. coli* cells at different stages of growth using a Coulter Counter*. They found that the size of cells in the logarithmic stage of growth was relatively constant at 0.70 μm^3. The size decreased by 10-15% upon reaching the stationary phase. This is a much smaller difference in cell size than that found by Hulsheger *et al*. (1983) and listed in Table 2.1. Zimmermann *et al*. (1974) also found that the form factor for both logarithmic and stationary phase cells was 1.13,

* A description of this device can be found in the glossary of this thesis.

which closely matches that found by Hulsheger *et al.* (1983).

Chapter 3

PULSED ELECTRIC FIELD TREATMENT

A high voltage electric field can cause permeabilisation of the cell membrane. This membrane permeabilisation can be either reversible or irreversible. Higher fields and longer exposure times cause irreversible membrane damage and cell lysis. Thus, high magnitude electric fields can be used in the disinfection or treatment of conducting liquids.

High voltage treatment technology is currently under much research and development. This technology is called Pulsed Electric Field (PEF) treatment and is mainly being applied to the treatment of liquid food such as milk and fruit juice [Castro *et al*. 1993, Grahl and Markl 1996, Jeyamkondan *et al*. 1999, Mazurek *et al*. 1995, Palaniappan and Sastry 1990, Qin *et al*. 1994 & 1995, Vega-Mercado *et al*. 1997, Wouters *et al*. 1999]. Only a few researchers have applied PEF treatment to water [Mizuno and Hori 1988, Schoenbach *et al*. 1997a].

The PEF treatment involves the charging and discharging of high voltage capacitors. The stored energy in the capacitors is transferred to the liquid by triggering a high voltage switch. PEF waveforms are a series of monopolar or bipolar pulses of varying frequency with pulse width usually between 10-800μs. These pulses can be an exponential decay or can be made square by use of a pulse forming network. The peak electric field strength for these pulses is usually between 8 - 40 kV/cm.

PEF treatments have been extensively tested on a range of bacteria. The results of these investigations have shown that it is possible to reduce the concentration of living cells by 1 to 9 orders of magnitude [Mazurek *et al*. 1995, Wouters *et al*. 1999]. These experiments found that the level of reduction depends on many factors including medium temperature, conductivity, field strength, pulse duration, number of pulse applications, cell species and growth stage [Jeyamkondan *et al*. 1999].

3.1 Factors that Influence PEF Effectiveness

Electric field strength, pulse duration and number of pulses have the greatest effect on the survival ratio [Hulsheger *et al*. 1981], and nearly all authors have examined the effect of these. Other factors that have been examined are discussed below.

3.1.1 Pulse shape

Qin *et al*. (1994) studied the effects of different voltage waveforms on the inactivation rate of bacteria and yeasts. They found that square shaped pulses were more effective than exponential decay pulses. Oscillatory decay pulses were the least effective because the electric field is continually changing and is only above the critical

value for short periods. They also found that bipolar pulses are more effective than monopolar pulses. The sudden reversal in electric field orientation applies further stress on the membrane due to the direction of movement of the charges [Qin *et al.* (1994)]. However this is still a subject of investigation.

3.1.2 Medium temperature

Hulsheger *et al*. (1981) studied the effects of medium temperature on PEF effectiveness. Increasing the medium temperature increases the amount of inactivation. This is because the breakdown transmembrane potential is decreased, in addition to possible thermal-injury effects. These findings have been verified by Wouters *et al*. (1999).

3.1.3 Medium conductivity

Most cited experiments have been undertaken either on liquid foods or a salt electrolyte. These types of liquids have a higher conductivity than that of water. Several authors have found that PEF treatment is more effective on lower conductivity fluids [Jayaram *et al*. 1992b & 1993, Jeyamkondan *et al*. 1999, Matsumoto *et al*. 1991, Wouters *et al*. 1999]. Jayaram *et al*. (1993) argued that PEF is more effective on lower conductivity fluids because of the larger difference in ionic concentration between the suspension and cell cytoplasm. The large ionic gradient facilitates an increased flow of ionic substances across the membrane, which weakens the membrane structure and makes it more susceptible to PEF treatment. Mizuno and Hori (1988) used PEF on *Saccharomyces cerevisiae* (yeast cell) and vegetative *Bacillus natto* cells suspended in deionised water of conductivity 6 μS/cm. The treatment was effective on both organisms. The reduction in survival ratio for the yeast cells was up to 6 log.

3.1.4 Microorganism Type

PEF treatment has been tested on a wide range of microorganisms. The effectiveness of the treatment varies for different organism types and structures. Sale and Hamilton (1967) reported that yeasts were more sensitive to the treatment than were vegetative bacteria. This is contrary to the results of Hulsheger *et al*. (1983) who found that yeasts were less sensitive, but in agreement with Qin *et al*. (1994). Yeasts are larger than most bacteria and therefore a greater transmembrane potential can be generated across the membrane under identical field strength. Therefore they may be more susceptible to PEF.

A few authors have examined the effects of PEF treatment on both gram-negative and gram-positive bacteria [Hulsheger *et al*. 1983]. Gram-positive bacteria are less sensitive to electric fields than gram-negative bacteria.

Many authors have found that bacterial endospores are reasonably resistant to high voltage PEF treatment [Matsumoto *et al*. 1991, Knorr *et al*. 1994, Grahl and Markl 1996]. However, more recent research has found that bacterial spores can be inactivated given the right conditions. Marquez *et al.* (1997) did some testing on spore forming bacteria. They found that spores of *Bacillus subtilis* could be reduced by more than 3-log upon application of 30 pulses of 50 kV/cm. Spores of *Bacillus cereus* could be reduced by 5-log upon application of 50 pulses of the same field strength. In both of these experiments the spores were nearly all inactivated since the initial concentrations were 3.4-log and 5-log for *B. subtilis* and *B. cereus* respectively. They found that

treatments at lower electric field strengths or with a lower number of pulses had little effect on the viability of spores. For *B. subtilis*, 15 pulses of 50 kV/cm reduced numbers by only 0.2-log. Thus the killing threshold is very sudden. Depending on treatment parameters, either all, or very little, of the spores are inactivated.

Mizuno *et al.* (1990) examined the effectiveness of PEF treatment on two different types of virus. They tested on Swine Vesticular Disease Virus (SVDV) and Equine Herpesvirus-1 (EHV-1). They reported a reduction in survival of SVDV at >8.16 log, and a reduction in survival of EHV-1 at >4.71 log. Upon observation under an electron microscope they found apparent damages to the genetic material inside the virus. However, there is no other cited literature to support these findings.

3.1.5 Cell Growth Stage

A few authors have looked into the effects of cell growth stage on the effectiveness of the PEF treatment [Hulsheger *et al*. 1983, Pothakamury *et al*. 1996, Schoenbach *et al*. 1997, Wouters *et al*. 1999]. All of them have found that to varying degrees, cells harvested in the growth phase are more susceptible to the electric fields than cells harvested in the late stationary phase. Pothakamury *et al*. (1996) looked into the effects of growth stage in some detail. They treated the bacteria *Escherichia coli* harvested at various stages of growth with high voltage PEF. They found that exponential-stage cells were more sensitive than stationary-stage and lag-stage cells to the PEF treatment. None of these authors offer any explanation or discussion for this observed difference. Most of the authors treat the finding as just another factor influencing the rate of cell inactivation.

3.2 Mathematical Model of the Survival Ratio

Hulsheger *et al*. (1981) empirically developed a mathematical model for the survival rate as a function of electric field and treatment time:

$$s = \left(\frac{t}{t_c}\right)^{[-(E-E_c)]/k} \tag{3.1}$$

where s is the survival ratio, t is the treatment time (product of number of pulses and pulse width), t_c is the critical treatment time, below which no reduction in survival occurs, E is the applied electric field strength, E_c is the critical electric field strength (below which no reduction in survival occurs), and k is a specific constant for a microorganism.

It is usually convenient to express the survival ratio on a logarithmic scale. In this case, Equation 3.1 may be rearranged to give:

$$-\log(s) = \frac{(E - E_c)}{k}.\log\left(\frac{t}{t_c}\right) \tag{3.2}$$

The logarithmic survival ratio is directly proportional to the electric field strength, E, and proportional to the logarithm of treatment time, t. This indicates that the electric field strength has more effect on the survival ratio than the treatment time. That is, in

order to achieve a greater reduction in survival, it is more efficient to increase the field strength rather than increase the treatment time.

The validity of Equations 3.1 and 3.2 have been verified by other authors [Grahl and Markl 1996].

3.3 Summary of PEF testing on Liquid Foods

A summary of the PEF testing performed on various liquid foods by cited references is shown in Table 3.1.

Foods	Researchers	Microorganisms	Log reduction in viability	Electric field (kV/cm)
Apple juice	Evrendilek *et al*. (1999)	*Escherichia coli*	5	30
Apple juice	Zhang *et al*. (1994b)	*Saccharomyces cerevisiae*	4	12
Apple juice	Qin *et al*. (1995)	*Saccharomyces cerevisiae*	6	50
Egg (liquid)	Martin-Belloso *et al*. (1997)	*Escherichia coli*	6	26
Milk	Reina *et al*. (1998)	*Listeria monocytogenes*	2.6	30
Milk	Dunn and Pearlman (1987)	*Escherichia coli*	3	21
Milk	Dunn and Pearlman (1987)	*Salmonella dublin*	4	18
Milk	Grahl *et al*. (1992)	*Lactobacillus brevis*	4.5	23
Milk	Grahl *et al*. (1992)	*Escherichia coli*	3	23
Milk	Gupta and Murray (1988)	*Psuedomonas fragi*	4.5	90
Milk	Grahl *et al*. (1992)	*Escherichia coli*	3	22
Milk (skim)	Martin *et al*. (1997)	*Escherichia coli*	2	45
NaCl buffer	Jayaram *et al.* (1992a)	*Lactobacillus brevis*	6	25
NaCl buffer	Gupta and Murray (1988)	*Salmonella typhimurium*	5	83
NaCl buffer	Hamilton and Sale (1967)	*Staphylococcus aureus*	2	27.5
NaCl buffer	Mizuno and Hori (1988)	*Saccharomyces cerevisiae*	3	17
Orange juice	Grahl *et al*. (1992)	*Saccharomyces cerevisiae*	5	7
Peptone (0.1%)	Ho *et al*. (1995)	*Pseudomanas fluorescens*	>6	10
Phosphate buffer	Matsumoto *et al*. (1991)	*Saccharomyces cerevisiae*	5	30
Phosphate buffer	Matsumoto *et al*. (1991)	*Escherichia coli*	5	40
Phosphate buffer	Hulsheger *et al*. (1983)	*Escherichia coli*	3	20
Phosphate buffer	Hulsheger *et al*. (1983)	*Klebsiella pneumoniae*	3	20
Phosphate buffer	Hulsheger *et al*. (1983)	*Pseudomonas aeruginosa*	3.5	20
Phosphate buffer	Hulsheger *et al*. (1983)	*Staphylococcus aureus*	3	20
Phosphate buffer	Hulsheger *et al*. (1983)	*Listeria monocytogenes*	2	20
Phosphate buffer	Hulsheger *et al*. (1983)	*Candida albicans*	4	20
Saline solution	Hamilton and Sale (1967)	*Escherichia coli*	2	19.5
Simulated milk	Zhang *et al*. (1994a)	*Escherichia coli*	9	70
Yoghurt	Dunn and Pearlman (1987)	*Saccharomyces cerevisiae*	3	18
Yoghurt	Dunn and Pearlman (1987)	*Lactobacillus brevis*	2	18

Table 3.1 Summary of PEF testing on liquid foods

This PEF testing has been undertaken by a variety of authors on different liquid media. The microorganisms tested include gram-positive and gram-negative bacteria and yeast. The log-reduction of these organisms is shown in the table and compared with the peak electric field strength. It can be seen from the table that there is a wide variation in results between the different references. For example, Grahl *et al*. (1992) achieved a 5-log reduction of the yeast *S. cerevisiae* using a peak electric field strength of only 7 kV/cm, whereas Dunn and Pearlman (1987) achieved only 3-log reduction on the same organism using a higher field strength of 18 kV/cm.

Chapter 4

DEVELOPMENT OF THE WATERISER

4.1 Introduction

A working model of a high voltage water purification device was designed and constructed for use in the domestic market. This development model device was to operate on a 240V, 50Hz, AC mains supply, delivering a flow rate of one litre per minute of purified water for drinking purposes. The maximum flow rate was limited to one litre per minute because of the deionising resin. A standard, commercially available deionising resin cartridge was used in this device. The device was designed to fit under a sink bench and be piped to a faucet mounted next to the sink.

4.2 Apparatus

A block diagram of the device is shown in Figure 4.1. There are five main sub-sections: the deionising resins, air trap, electrode chamber, high voltage source, and control electronics.

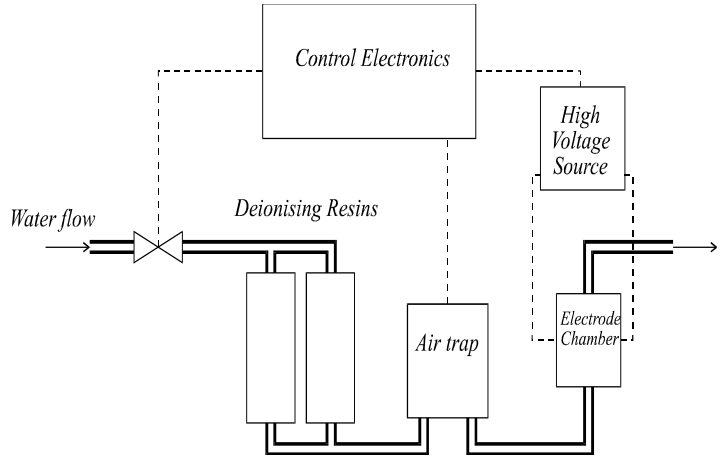


Figure 4.1 Purification process

4.2.1 Deionising Resins

The application of a continuous high voltage to the electrodes with water between them would normally require huge power levels due to the high electrical conductivity of the water. The water conductivity must first be lowered before high voltage treatment can be applied.

Electrical charge is carried through the water by ions. By removing these charged ions, the conductivity of the water may be reduced. Ions can be removed from solution by passing the solution through an ion exchange resin. The development model uses a mixed bed resin, which removes both anions and cations. The resin exchanges H^+ ions for cations and OH^- ions for anions (Figure 4.2). H^+ and OH^- ions combine to form water (H_2O).

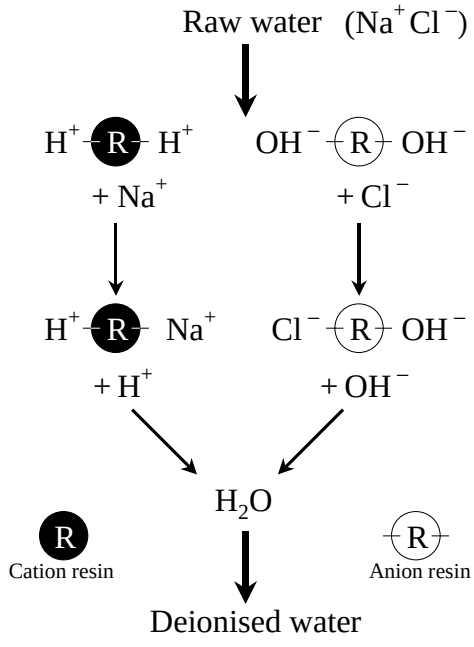


Figure 4.2 Mixed-bed water deionisation process

Larger industrial sized deionisation plants may separate the anion and cation beds. This separation enables easy regeneration of each resin once the resins have been exhausted. Separate bed resins do not provide such high quality water as a mixed bed resin. For this reason, a mixed-bed resin may be used as a final stage polisher to improve the output water quality. Most mixed bed resins cannot be regenerated and are discarded after use. Mixed bed resins that have anion and cation beads of different densities, can be separated and also regenerated.

4.2.2 Air Trap

Air bubbles in the water can cause problems in the electrode chamber. The electric field strength between the electrodes is higher than the breakdown strength of air. Thus air bubbles within the high electric field may breakdown and initiate arcing across the electrode surfaces. This arcing between electrodes leads to degradation of the electrode surface and causes electrical current surges that can damage various electrical components within the device. Air bubbles in the water can originate from the inlet water supply, initial installation of the device, when resin cartridges are replaced, or may be due to dissolved gases coming out of solution due to changes in water pressure throughout the system.

The function of the air trap is to remove any air bubbles from the water before they reach the electrode chamber. The water flows into and out from the trap from the bottom (Figure 4.3). The decrease in pressure and velocity of the water as it enters the trap due to its large volume causes any air bubbles to float to the top of the trap. When

the water level lowers due to a build up of air, the air trap float switch triggers a release of air via the solenoid valve.

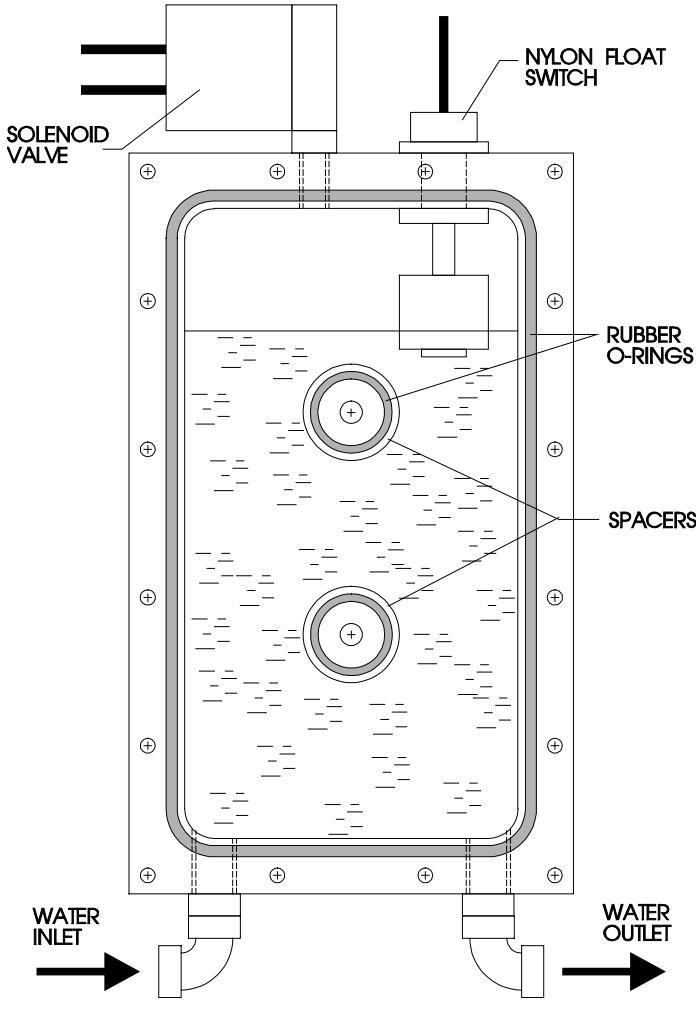


Figure 4.3 Air trap assembly

The main body of the air trap was machined out of ultra high density polyethylene. Transparent acrylic plates were screwed in to either side of the main body and sealed by use of large rubber o-rings. Two acetal spacers are necessary to prevent the acrylic sides from flexing when placed under full water pressure.

4.2.3 Electrode Chamber

The electrode chamber is where the disinfection process takes place. Bacteria and living organisms in the water experience a high electric field as they pass between the electrodes. This high electric field causes dielectric breakdown of the cell membranes, resulting in cell lysis. Throughout this thesis, *exposure time* is defined as the length of time a suspended particle is directly between the electrodes during treatment (ie. in the region of high field strength).

The electrode chamber was designed to produce a high electric field in the order of 30 kV/cm rms. This field strength is low enough to prevent the breakdown of water, but large enough to achieve a high rate of cell lysis. A small electrode gap was chosen in order to keep the voltage potential reasonably low to avoid design problems associated

with high voltage handling and safety. The electrode gap was thus chosen to be 2.0mm, with a corresponding potential of 6 kV AC.

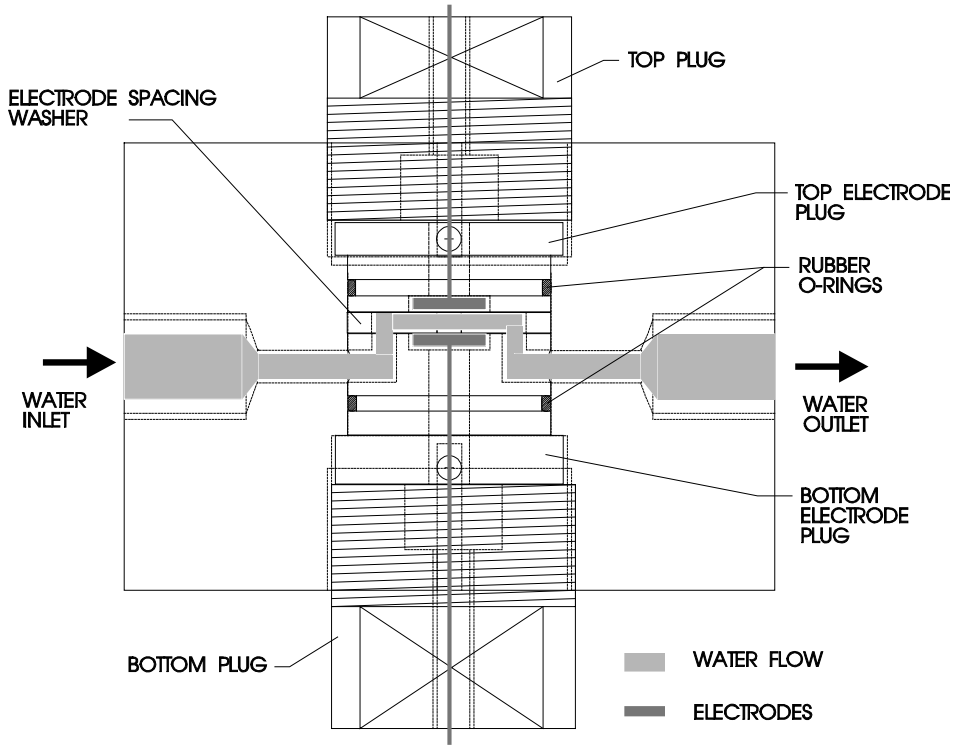


Figure 4.4 Electrode chamber assembly

A diagram of the assembled electrode chamber is shown in Figure 4.4. The electrodes consist of two square parallel titanium plates. Titanium was used due to its passivity (it minimises electrolysis effects) and strength. Parallel plate electrodes were chosen because they are easy to build on this small scale, and they produce a uniform field. The plates are square in order to minimise edge effects and for ease of assembly.

4.2.4 High Voltage Source

The high voltage across the electrodes is supplied by a custom-built 230V:6000V, 50Hz step-up transformer. The transformer is rated at a maximum of 200VA. The resistance of the water between a set of parallel plate electrodes may be calculated from

$$R_L = \frac{g}{\kappa A} \tag{4.1}$$

where g is the gap between electrodes (0.2 cm), A the area of the electrodes (1 cm^2) and κ is the conductivity of the water (µS/cm). Using Equation 4.1, the water resistance is 200kΩ at a water conductivity of 1µS/cm. The load power may be calculated from:

$$P_L = \frac{V_L^{\,2}}{R_L} = \frac{V_L^{\,2}\kappa A}{g} \tag{4.2}$$

where V_L is the load voltage (6,000V rms). The power required to treat water with conductivity 1μS/cm is thus 180W. Assuming that the transformer is 90% efficient, then this is the maximum conductivity the 200VA transformer can continuously treat.

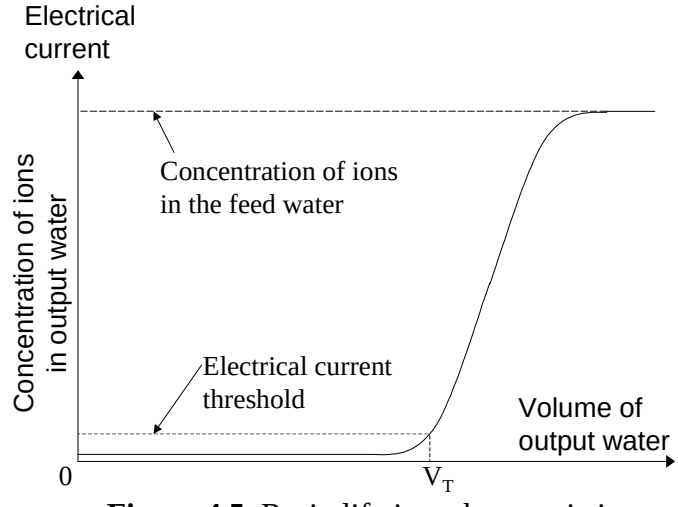


Figure 4.5 Resin lifetime characteristics

A typical mixed-bed deionising resin will have a finite lifetime with characteristics similar to that shown in Figure 4.5. The initial output water quality will be high and remain relatively constant. The electrical current or power required to disinfect the water at these early stages will be low. As the resin becomes exhausted it no longer has the capacity to remove all ions. The water conductivity begins to rise and will do so in a linear fashion until the bed is completely exhausted and the concentration of ions in the output water is equal to that of the inlet water. The electrical current threshold is set at some level above the normal current level and is used to indicate when the resin becomes exhausted and needs replacing.

4.2.5 Control Electronics

The electronics control the overall process. Analogue signals from the load voltage and current sensors are converted to digital signals. A discreet digital logic section controls the water flow, air trap release valve and high voltage relay timing.

A visual LED display is mounted on the unit. In addition, a momentary push-button switch is used to disable the high voltage. The high voltage must be disabled whenever the device is to be installed, to allow the system to fill with water. If the high voltage is enabled when there is no water between the electrodes, electrical breakdown and arcing will occur.

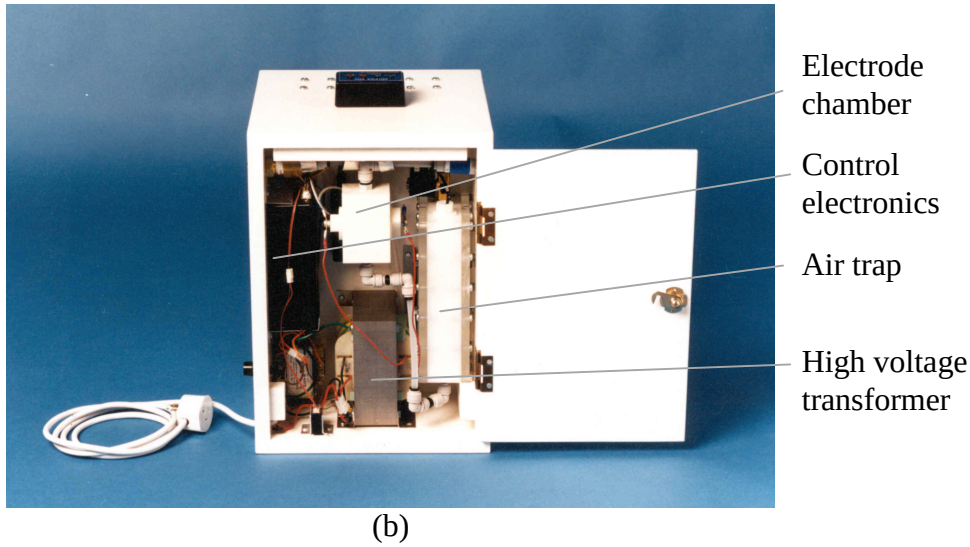
The device monitors the load voltage directly, and if the voltage drops below 80-90% of full voltage, the device will switch off and an alarm will be displayed. This alarm can occur if the load is drawing too much power (the conductivity of the water is high) or continuous arcing is occurring (air bubbles have entered the system).

The device also monitors the primary current into the transformer. When the current rises above the resin threshold this indicates that the deionising resins are becoming exhausted and will require replacing. The device switches off and issues a warning to replace the resins.

The assembled development model of the water purification device is shown in Figure 4.6. The deionising resin cartridges are mounted at the front of the unit for easy

access. The high voltage, electrical components and electrode chamber are enclosed within the unit for safety reasons.

(a)



(b)

Figure 4.6 Completed development model showing (a) deionising resin cartridges and (b) internal components

4.3 Biological Testing

The effectiveness of the high voltage was tested on bacteria. In order to measure this for the demonstration model, the deionising resins were bypassed. The bacteria were suspended in deionised water and passed through the electrodes directly. This was to avoid the filtering effect that deionising resins have on bacteria. The small negative charge inherent on the surface of bacteria tends to cause a high percentage of them to be removed from solution by the resins.

Serratia marcescens are small Gram negative bacteria. They consist of rod shaped cells either straight or curved, not exceeding 0.5μm in width. *S. marcescens* was chosen as a suitable bacteria for experimentation due to its small size and therefore smaller transmembrane potential for a given electric field (Equation 2.4). Thus, they should be

more difficult to lyse than larger bacteria. *S. marcescens* is also pigmented red which easily distinguishes it from other bacteria. Also, it does not normally cause illness in humans.

The bacteria *S. marcescens* were grown in standard nutrient broth and incubated at 30°C overnight (15 Hrs). This corresponds to the early stationary growth phase (refer to Chapter 14). The bacteria were resuspended in deionised water and run through the development model electrodes while varying the flow rate and peak voltages. The conductivity of the resulting solution for each experiment was in the range 1.0 - 1.4 μS/cm. Control samples were taken with the high voltage switched off. The bacteria concentrations of the controls and disinfected samples were measured by means of the serial dilution method. The bacterial counts from the samples were compared to that of the controls to establish the survival ratio. The survival ratio is defined as N/N_0, where N is the number of viable cells after treatment, and N_0 is the initial number of viable cells. In these experiments the initial number of cells, N_0, was between 20,000 and 100,000 cells per cm^3.

A graph of the survival ratio of *S. Marcescens* after being treated by the high voltage can be seen in Figure 4.3. The 21 kV/cm results were taken from a single experiment. The 27 and 30 kV/cm results were taken from a number of repeated experiments over different days.

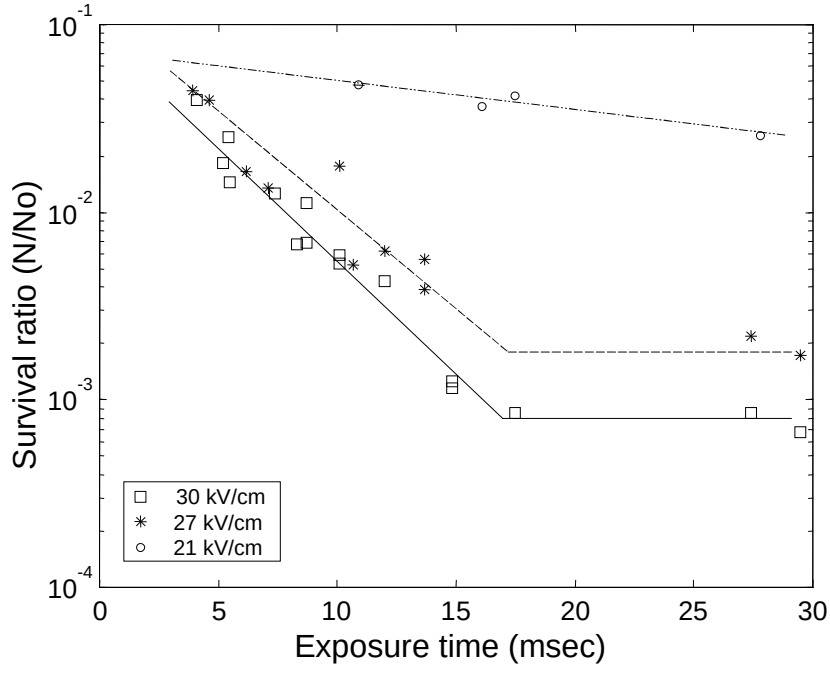


Figure 4.3 Treatment of *S. marcescens*

For the experiments using high field strengths, 27 and 30 kV/cm respectively, the survival ratio reaches a lower limit. The survival ratio of bacteria decreases exponentially with increasing exposure time up to around 17ms. When the exposure time is increased above this value, there is no significant change in the survival ratio. The reason for this effect was unclear at the time of the experiments. However, some possible answers for this were later discovered and are discussed in Chapter 14.

The most efficient use of the disinfection unit occurs at an exposure time of 17ms. This equates to a flow rate of 700 ml per minute. At this flow rate the survival ratio of *S.*

marcescens is less than 0.001 at an electric field strength of 30kV/cm. This is equivalent to greater than 99.9% of bacteria being lysed.

4.4 Energy Use

The high voltage method of water disinfection is energy efficient when compared to some other technologies. The device requires a total of 40-60W of power to disinfect a steady flow of 700 ml per minute. This implies that the actual conductivity of the deionised water is less than the 1μS/cm used to calculate the maximum rating of the transformer (Section 4.2.4). The increase in temperature of the water remains unchanged. The method uses much less energy than boiling the water. At a nominal operating power of 60W and an exposure time of 17 msec, the energy used by the high voltage disinfection is approximately 1 J/ml. The energy required to raise the temperature of water from 20^{o}C to 100^{o}C ($\Delta E=\Delta T.m.c$, where m is the mass and c is the specific heat capacity of water, c= 4.19 J $g^{-1}(C°)^{-1}$) consistent with boiling water is 335 J/ml.

4.5 Conclusions

A demonstration model of a high voltage purification device has been developed for use in the domestic drinking water market. The device has been successfully tested. The device rendered over 99.9% of *S. marcescens* non-viable, operates on mains water and 230V electrical supplies, requires between 40 and 60W of power, and disinfects water at an optimum flow rate of 700 ml/min.

The conductivity of the liquid must be low to keep the power requirements to acceptable levels. This is achieved by the use of a mixed-bed deionising resin prior to disinfection. Air bubbles in the liquid, which can cause arcing and degradation of the electrode surfaces, are removed by use of an appropriate air trap.

Due to the success of this device, patent rights have been filed with a view to developing this technology in the domestic and industrial markets.

Chapter 5

COMMERCIALISATION OF THE WATERISER

5.1 Introduction

A working model of a domestic water purification device has been constructed and tested (Chapter 4). The next step was to commercialise the device ready for marketing as a domestic water purification unit. This chapter outlines changes in the device that were required for commercialisation and industrial production. It describes these changes and compares the performance of the new commercial prototype with the original development model.

5.2 Commercial Prototype Design

The initial development model (Chapter 4) was redesigned to allow for commercial production. A block diagram describing the new process is shown in Figure 5.1.

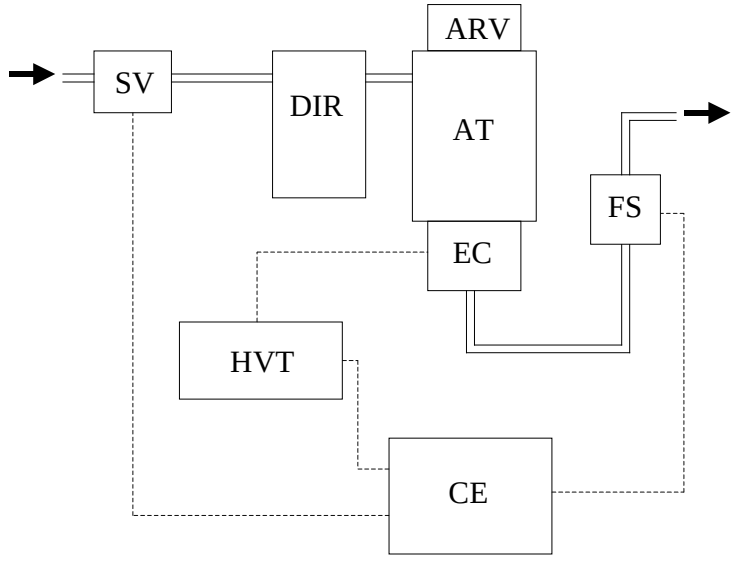


Figure 5.1 Commercial prototype block diagram

The main design changes were the combination of the air trap (AT) and electrode chamber (EC), the use of a mechanical air release valve (ARV), a change in electrode shape and construction, a change in the high voltage transformer (HVT) specifications, and the incorporation of an electrical/mechanical flow switch (FS). The control electronics (CE), solenoid valve (SV) and deionising resin (DIR) remained the same as

those used in the development model. The components that were changed are described in more detail in Sections 5.2.1 - 5.2.4.

5.2.1 Electrode and air trap combination

The electrode chamber and air trap were incorporated into one complete unit, as shown in Figure 5.2.

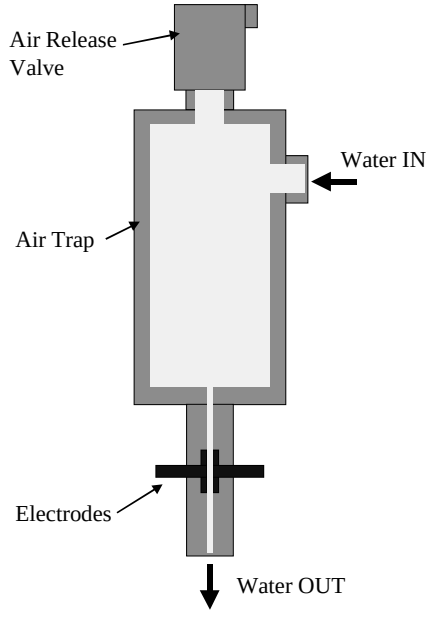


Figure 5.2 Electrode / air trap construction

This combination was mainly for ease of manufacture. The air trap and electrode chamber were both constructed from plastic and lend themselves to being injection moulded. It was convenient to combine these components and thus avoid the need for two separate chambers with associated plumbing attachments. The combined AT/EC chamber was injection moulded in two pieces that were then heat jointed together.

Another important reason for the air trap and electrode chamber combination is that the air trap is a necessary prerequisite to the electrode chamber. If air bubbles are allowed to reach the electrode chamber, electrical arcing and electrode degradation will occur. The air trap is designed around the same operating characteristics as the electrode chamber. The two components are matched for any given flow rate.

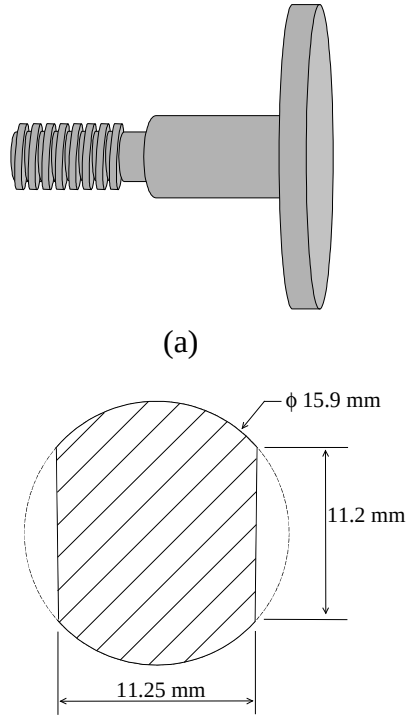
The air release valve (R88/1,Giacomini, Italy) is mechanical and screws in to the top of the air trap. This simplifies the device by automating the release of air and avoids an expensive solenoid valve and water level sensor.

5.2.2 Electrode Dimensions and Construction

The dimensions of the electrodes are different from the initial development model. A new set of electrodes has been designed to be incorporated into the injection moulding process and allow for mass production. The electrode face was circular to allow for rotation in the moulding process. This removed the requirement to align square electrodes inside the mould.

A sketch of an electrode is shown in Figure 5.3(a), showing its shape. It was machined out of 316T stainless steel. This is different to the initial development model that uses titanium. Stainless steel is more common, easier to work with, and less expensive than titanium. This grade of stainless steel does not degrade significantly over time, as found by experimental observation. A unit has been trialed for over 6 months without a significant change in performance. The combined air trap and electrode chamber may be replaced if there was a degradation in performance over time. Material costs for this component are minor relative to the cost of the overall unit.

Fig 5.3(b) shows the effective electrode area in contact with the water. This area is calculated to be 1.62cm^2. The electrode area is higher than that of the development model (1.0 cm^2). However, the exposure time at a given flow rate is approximately the same since the electrode gap has been decreased.



(a)

(b)

Figure 5.3 Electrode (a) shape (b) dimensions in mm

5.2.3 High Voltage Transformer

The gap between the electrode surfaces has been reduced from 2mm to 1.5mm. This allows the voltage magnitude to be slightly lowered, easing the design of the high voltage transformer. In order to obtain a similar electric field strength of 30 kV/cm r.m.s., the voltage magnitude applied to the electrodes is 4.75 kV r.m.s. A suitable transformer with a 240:4750 voltage ratio was designed and manufactured by an existing transformer winding company. The design is suitable for large scale automated production.

5.2.4 Housing Design

The housing for the demonstration model was made from painted wood. This is not suitable for commercial production. The commercial prototype housing was initially

constructed from fibreglass. The housing would ultimately be made out of moulded plastic once manufacturing numbers were high enough to warrant initial die set-up costs.

The complete commercial prototype is shown in Figure 5.4.

Figure 5.4 The commercial prototype 1LPM device

5.3 Performance of the Commercial Prototype

The commercial prototype electrode chamber and air trap combination was found to work well. It was found to be more robust to the problem of air bubbles and electrode stability. It exhibited less arcing and was able to better withstand the injection of large amounts of air bubbles upstream.

The commercial prototype is much simpler to manufacture. The electrode chamber in the initial development model was machined out of solid plastic with five different parts and rubber o-rings to prevent water leakage. The new EC/AT combination may be easily injection moulded and produced quickly in mass quantity.

The costs of producing the new EC/AT combination would be much lower. For an initial run of 1000 units the cost per piece is estimated at $1/50^{th}$ the cost of producing the separate development model pieces.

The commercial prototype electrode chamber was tested on the bacteria *Serratia marcescens,* using an identical method as explained for the development model (Chapter 4). A comparison of results is graphed in Figure 5.5. The points shown for the commercial prototype are the results from two similar experiments done on different days. It can be seen that the commercial model provides a similar disinfection characteristic to the development model. The slight general decrease in kill rate may be attributed to the change in electrode dimensions and associated tolerances. The similarity in disinfection characteristic suggests the biological performance of the device is not overly compromised by the change in design parameters listed in Section 5.2.

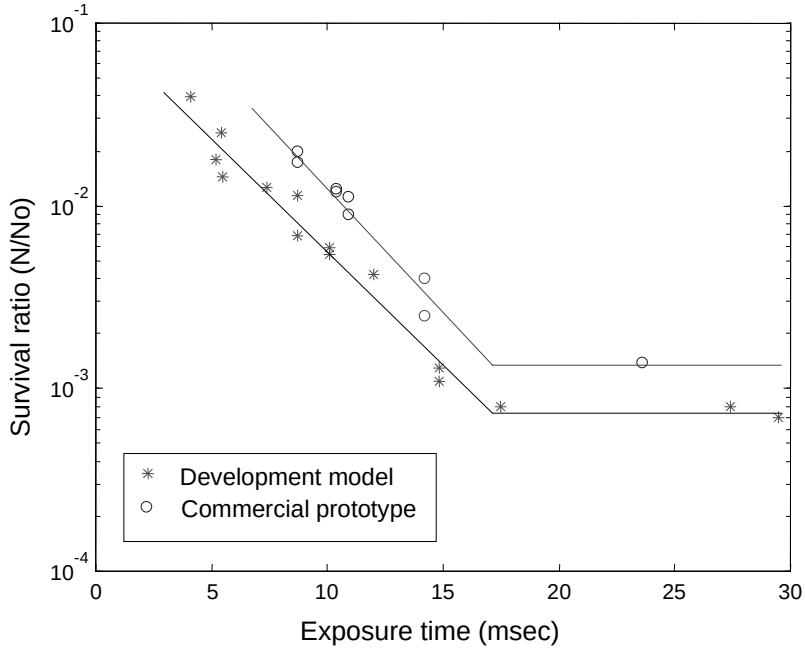


Figure 5.5 Survival ratio of *Serratia marcescens* for commercial prototype, compared with initial development model

5.4 Conclusions

A commercial prototype of a high voltage water purification device has been constructed. This device has a one litre per minute flow rate and is designed for use in domestic households.

Critical design parameters had to be changed from the initial development model to allow ease of manufacturing for the commercial prototype. These changes were in the electrode material, electrode shape and dimensions, high voltage magnitude and the air trap and electrode chamber combination. Despite these design changes, the commercial prototype had a similar disinfection performance as the demonstration model.

The combination of the air trap and electrode chamber into one component also increased the electrical robustness of the device. The whole component may be injection moulded, decreasing the cost and time of manufacture.

Chapter 6

EFFECTS OF HIGH MAGNITUDE ELECTRIC FIELDS ON THE VIABLITY OF GIARDIA INTESTINALIS

6.1 Introduction

In this chapter, the effect of the 50Hz device on *Giardia intestinalis*, a parasitic protozoan commonly found in water supplies, is reported. *Giardia* organisms can exist in two different forms; the cyst form outside of the host, and the trophozoite form when inside the host. In the cyst form, the parasite is dormant and can survive outside the host for up to 56 days [deRegnier *et al*. 1989]. When it enters a host, the cyst lodges in the small intestine where it excysts into two trophozoites. These organisms multiply rapidly and cause symptoms such as explosive, foul smelling diarrhoea, stomach cramps, bloating, dehydration, nausea, and weight loss.

Infected hosts pass *Giardia* cysts in their faeces. When water supplies become contaminated, cysts can be disseminated to infect other hosts. Because of the prevalence of *Giardia* cysts in water sources and the potential for disease, the search for an effective disinfection device is paramount.

A *Giardia* cyst has a wall that encases a membrane about the two trophozoites. This is a different structure to the phospholipid bilayer of bacteria, upon which electroporation concepts have been developed. The cyst wall consists of a tightly packed layer of chitinous elements and is of the order of 0.3-0.5μm in thickness [Kulda and Nohynkova 1978, Sheffield and Bjorvatn 1977]. This makes the cyst potentially much more difficult to kill. The 50Hz device is tested to determine whether cysts suspended in water can be made non-viable by the disruption of the cyst wall and membrane. Its performance is compared to that of PEF treatment. An electric fence unit generates a high voltage waveform similar to that used in conventional PEF applications.

6.2 Materials and Methods

Two sets of experiments were undertaken on *Giardia* cysts, which were spiked into water. The first experiment used an electric fence energiser to apply a high voltage pulse of short duration to a static volume of solution, similar to commercial electroporation devices but with a higher voltage. The second experiment used equipment from the 1LPM Wateriser (Chapter 4).

6.2.1 Electric Fence Energiser

The electric fence energiser test set up is shown in Figure 6.1. The energiser type was a 'Maxi Grazer' manufactured by Staffix Electric Fencing, New Zealand. The energiser was powered by a 12V dc battery.

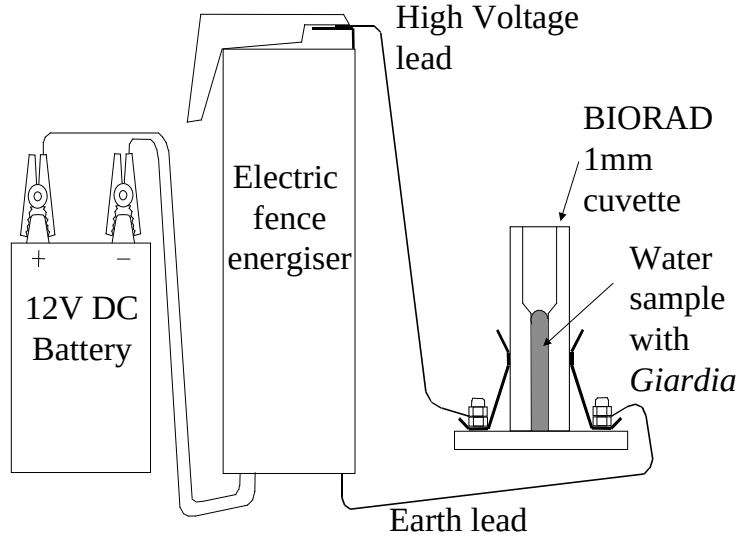


Figure 6.1 Electric fence energiser test set-up.

The energiser circuit incorporates a dc to dc converter to step up the voltage from 12V to 320V dc. The capacitive DC bus is discharged into the primary winding of a step-up pulse transformer by the triggering of a silicon controlled rectifier (SCR). The typical voltage waveform induced across the load by the secondary winding of the pulse transformer can be seen in Figure 6.2. The peak voltage is about 4kV, with a rise time between 2 and 5 μs, and a decay time constant of 10 μs.

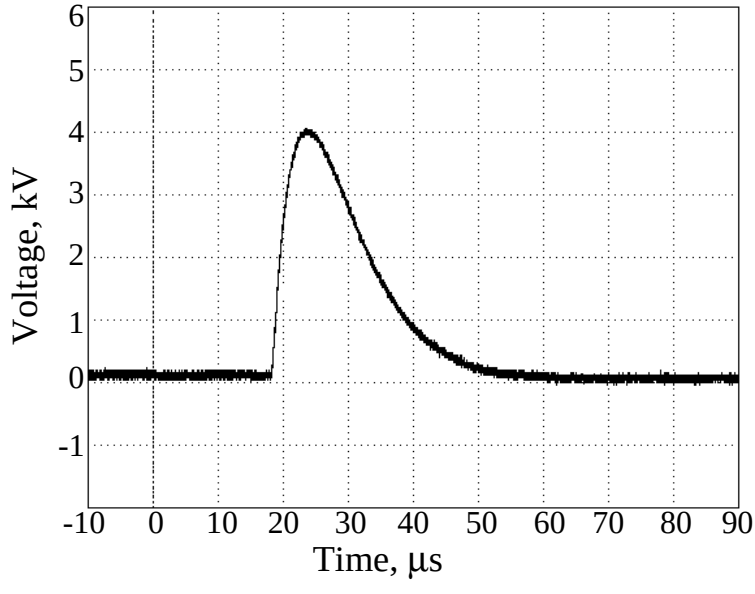


Figure 6.2 Typical voltage waveform across the water in the 1mm cuvette.

The water sample was placed in a cuvette (BIO-RAD Laboratories, 2000 Alfred Nobel Drive, Hercules, California 94547) with a 1 mm spacing between the electrodes. This gave peak electric field strength across the water of 40kV/cm. This value is similar to the peak field strength used in the 50Hz treatment model.

When the electric fence energiser was connected to the battery a series of 4kV pulses were applied to the electrodes. The energiser was set to 'slow' pulse repetition. The duration between each pulse was measured to be 2.6 seconds. The number of consecutive pulses used in this experiment was five.

6.2.2 50Hz Treatment Model

The experimental equipment was set-up as shown in Figure 6.3. The two cylindrical storage containers A and B hold up to 2.2 litres of liquid. They were constructed out of stainless steel to withstand internal pressures of up to 350 kPa. The spiked deionised water was put into container A and the disinfected output water was collected in container B.

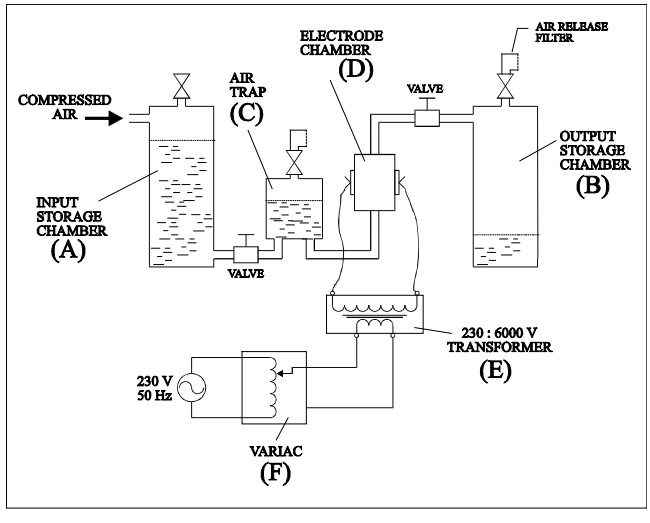


Figure 6.3 50Hz treatment set-up.

The electrode chamber (D) is identical in construction to the electrodes used by the 1LPM Wateriser (Chapter 4). The electrodes consist of two parallel titanium metal plates, each being 1cm square, separated by a gap of 0.2cm. The electrodes are mounted in a solid block of acetal plastic.

The air trap (C) removes any air bubbles present in the water before they reach the electrode chamber. This prevents arcing between the electrodes. The air trap was constructed from polyethylene with transparent acrylic sides. A manually operated air release valve is mounted at the top of the air trap.

High voltage is applied to the electrodes from a 50Hz step-up transformer (E). The primary of this transformer is supplied by a variac or autotransformer (F) connected directly to a single phase, 240V, 50Hz mains supply. The high voltage supply can be adjusted to obtain an output of 6kV that is fed directly to the electrodes. The resulting electric field strength across the water between the electrodes is 30kV/cm r.m.s.

6.2.3 Biological Test Procedure

For both experiments, viable *Giardia* cysts were obtained from the faeces of an infected calf, isolated, washed and resuspended in Hanks Balanced Salt Solution (HBSS). The concentration of cysts was approximately 10^5 per 100µl, estimated using a haemocytometer.

For the electric fence energiser experiment, 50µl of cyst solution was placed between the electrodes in the cuvette. Five consecutive 40 kV/cm electrical impulses were then applied across the electrodes. The treated sample was then collected and analysed for cyst viability.

For the 50Hz treatment model, 100µl of cyst solution (approximately 10^5 cysts) was suspended in 2L of deionised water and added to the input storage chamber. The conductivity of the resulting solution was measured at 1.2µS/cm. Compressed air was used to force the spiked water through the system at a flow rate of 0.7L/min, allowing the cysts to be in the electric field for an average of 19msec. The high voltage was applied to the electrodes immediately prior to the spiked water reaching the electrode chamber. This ensured that no cyst passed through the electrodes without experiencing the high electric field. Approximately 1L of spiked deionised water was passed between the electrodes and collected in the output storage chamber (B, Figure 6.3). The water flow and high voltage were then switched off simultaneously. The disinfected sample in the output storage chamber (B) and the untreated control sample (water remaining in the input storage chamber (A)) were then collected and the cysts analysed for viability.

The two samples (treated and control) were filtered through 0.22µm pore size membrane filters (Millipore Corp, MA, USA) using a vacuum manifold (Hoeffer model FH 255V, Hoefer Scientific Instuments, San Fancisco, CA, USA). The filters were suspended in 50ml Hanks Balanced Salt Solution (HBSS) and mixed by vortex to dislodge the cysts from the filter surface. The cysts were collected by centrifugation at 1050×g for 10min and resuspended in 100µl Hanks Balance Salt Solution (HBSS).

6.2.4 Assessing the Viability of Cysts

Cyst viability was assessed by the uptake of the fluorogenic dye 4'-6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) using a modification of the method reported by Campbell *et al*. (1992). 100µl of non-treated and electroporated cyst suspensions were added to 1ml of acidified Hanks Balanced Salt Solution (HBSS, HCl to pH 2.75) and incubated at 37^{O}C for 10min. The cysts were washed twice in 1ml non-acidified HBSS and resuspended in 100µl of non-acidified HBSS. 10µl of DAPI (2mg/ml) and 10µl of PI (1mg/ml) were added to the cysts and the suspension was incubated at 37^{O}C for 30min. Following incubation with the fluorogenic dyes, samples were incubated with 10µl of FITC (fluorescein isothiocyanate) labelled anti-*Giardia* cyst monoclonal antibody and incubated at 37°C for 30min in the dark. The cyst suspensions were washed twice in 1ml HBSS to remove unincorporated DAPI, PI and FITC-labelled anti-*Giardia* cyst monoclonal antibody.

10µl aliquots of cyst suspensions were viewed under epifluorescence with a Litz microscope equipped with the appropriate UV filter blocks [Campbell *et al*., 1992]. *Giardia* cysts were assessed for viability microscopically by the inclusion/exclusion of the fluorogenic dyes at 400× magnification (Figure 6.4a and 6.4b). The number of viable and non-viable cysts were enumerated in both the control and treated samples as

described by Campbell *et al*, (1992). Although 10^5 cysts were used in the treatment process, not all of the cysts were counted. Hence, to determine the initial proportion of cysts viable prior to high voltage treatment, 8 random microscopic fields at 400× magnification were counted. This procedure was repeated following the high voltage treatment.

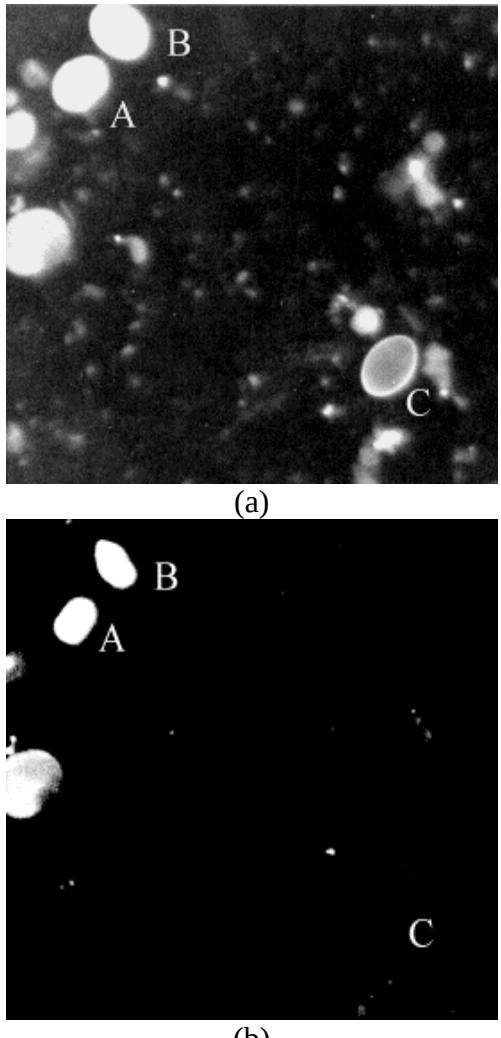


Figure 6.4 Images from fluorescence microscope showing the difference between dead and live cysts. (a) Results from the PI stain showing the stain has entered the dead cysts A & B, but has been excluded from the viable cyst C. (b) Results of the DAPI stain: cysts A & B are brightly fluorescent indicating they are dead (the nuclear material is distributed through the whole cell). Cyst C is viable.

6.3 Results

The treatment of *Giardia* cysts by the electric fence energiser is shown in Table 6.1. The viability of the *Giardia* cysts in the control was low, being only 15%. The low viability of the cysts may have been due to a poor faecal sample or length of time in storage before treatment. After treatment with five consecutive voltage pulses, the viability of cysts was about 2%. The electric field energiser treatment did indicate that

Giardia cysts could be rendered non-viable by the application of pulsed voltage sources commonly used in electroporation systems although some cysts remained viable.

	Untreated (control)	Treated
Cysts examined	100	100
No. viable	15	2
No. non-viable	85	98

Table 6.1 Viability of *Giardia* cysts (Electric fence energiser).

A visual examination of the *Giardia* cysts treated by the electric fence energiser showed that some cyst wall disruption had occurred. Cell wall fragments were seen suspended in the fluid.

For the 50Hz treatment model, an examination of 8 random microscopic fields at 400× magnification following fluorogenic vital dye staining showed that 96.6% of the cysts were viable prior to treatment (Table 6.2). This was a different source of cysts from that used in the electric fence experiment. Following high voltage treatment all the cysts were rendered non-viable.

	Untreated (control)	Treated
Cysts examined	408	1131
No. viable	396	0
No. non-viable	14	1131
% viable	96.6	0

Table 6.2 Viability of *Giardia* cysts (50Hz treatment model).

In the 50Hz treatment, an increase in the proportion of cyst wall debris was detected, as compared to the electric fence energiser treatment. This suggests that many of the *Giardia* cysts had been physically disrupted (Figure 6.5). The 'voltage × application time' product is much higher in the 50Hz experiments than for the pulsed waveforms. The pulsed waveform is much less severe on the cyst wall because of its very short duration.

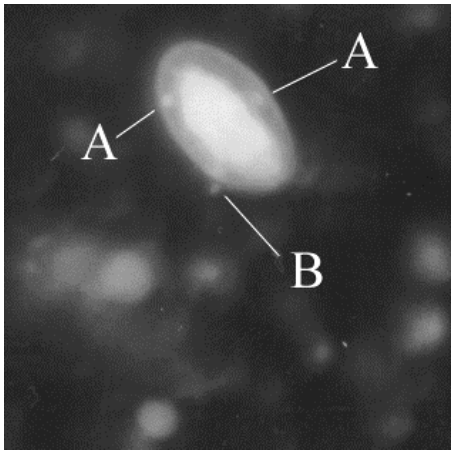


Figure 6.5 Treated *Giardia* cyst showing spots (A) and finger-like protrusion (B) through the cyst wall

6.4 Discussion

When *G. intestinalis* cysts suspended in deionised water are exposed to high magnitude electric fields, the cyst walls are disrupted. This leads to a loss of cyst viability.

No viable *Giardia* cysts were found on samples that had passed through the 50Hz high voltage treatment (the relative number of non-viable cysts is greater than 99.9% as determined by counting the number of viable and non-viable cysts in both treated and non-treated (control) samples). A visual examination of the high voltage treated cysts suggested that the outer cyst wall had been disrupted. Further, examination of non-viable cysts by diffraction interference contrast microscopy showed that the cyst wall had collapsed and the internal trophozoite structures could be seen embossed on the outside of the cyst wall. The cyst membrane was also seen detached from the inner cyst wall.

Some cysts were viable after treatment with a high magnitude electroporation device (electric fence energiser). Although there was a detected decrease in cyst viability, the electric fence energiser was not as effective a method for disinfecting cysts. This was due to a lower voltage pulse duration, and hence lower energy, delivered to the cyst wall.

Chapter 7

DESIGN OF A 10 LITRE-PER-MINUTE DEVICE

7.1 Introduction

In Chapters 4 & 5 the successful development of a one litre-per-minute (1LPM) water treatment device has been documented. This device is designed for use in domestic drinking water supplies. The deionising resin cartridges used in the device are what determine the maximum flow rate. The small resin cartridges are specified for a maximum flow of 1 LPM. The overall device has been designed to allow for mass production and distribution into these household markets.

Other commercial and industrial processes require much larger volumes of purified water, and as such the device needs to be scaled up to meet these applications. This chapter details the design of a 10 LPM device (e10) which is the first step in the scale up process. The changes in design that are required for these larger flow rates are detailed.

7.2 Component Changes Required for Larger Flow Rates

The 10 LPM device is designed around the same principle as the 1 LPM device. The block diagram of components is the same as that in Figure 5.1. Components need to be scaled up to suit the larger flows. A brief description of each component is given in this section.

7.2.1 Air Release Valve (ARV)

This is identical to the one used in the 1 LPM commercial model. It is a mechanical float valve that releases built up air in the air trap.

7.2.2 Air Trap (AT)

The one litre per minute (1 LPM) commercial prototype incorporated the air trap and electrode chamber into one integrated component. This integration allowed the component to be easily mass produced (injection moulded) and increased the electrical stability and robustness of the device. Hence, this idea was followed in the design of the larger flow 10 LPM water disinfection device.

The size of the air trap was increased proportionally to allow for the faster flow. The larger size means an increased capital cost of setting up an injection mould for this component. For the initial devices, a standard size PVC pipe system was used (65mm Class D pressure pipe). Caps were attached to either end of a 0.5m length of pipe. Entry

and exit holes were drilled and tapped. A diagram of the air trap assembly is presented in Figure 7.1. The air trap was mounted in an upright position. The water inlet was on one side of the pipe, the air release valve mounted at the top, and the electrode chamber attached beneath.

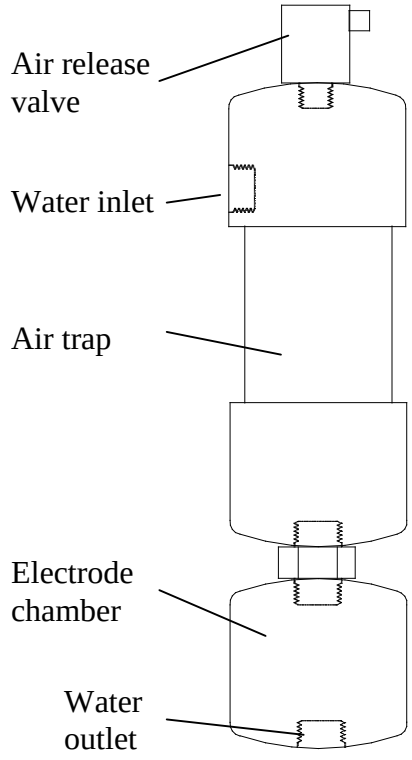


Figure 7.1 Air trap and electrode chamber assembly

7.2.3 Control Electronics (CE)

The control electronics are similar in function to the 1 LPM electronics. The electronics consist of a power board and control board. The high voltage transformer is turned on using a 40A triac.

7.2.4 Deionising Resin (DIR)

The deionising resin used for the 10 LPM device was the Ionmiser20 (US Filter New Zealand, Auckland, New Zealand). This has a maximum flow rate of 12 LPM, and a resin capacity of 25L. It is capable of deionising 10,000 litres of inlet water with conductivity 130 μS/cm (equivalent to the Christchurch water supply).

7.2.5 Electrode Chamber (EC)

The 1 LPM device uses a small parallel plate electrode system. Parallel plates are not practical for larger flow rates due to the large size and dimensions that would be required. Also, edge effects occur at the side edges of parallel plate electrodes as shown in Figure 7.2.

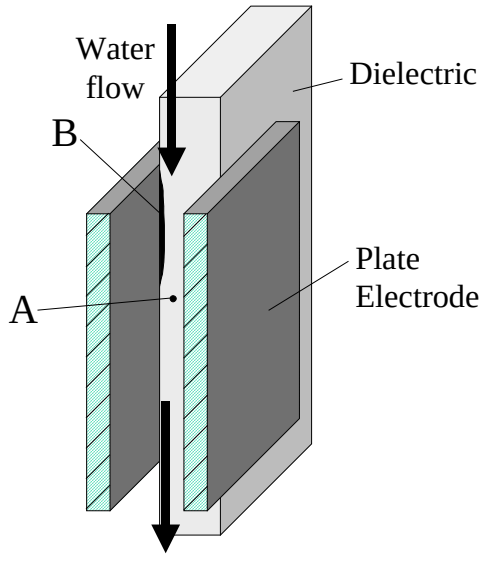


Figure 7.2 Diagram of edge effects due to plate electrodes showing (A) Region of low electric field strength at dielectric / water boundary (B) Imperfections in dielectric / electrode boundary may cause a small amount of water to pass between the dielectric and electrode.

At these edges two different problems may occur that would lower the effectiveness of lysing microorganisms that pass through. The first problem is at the water / dielectric boundary where the dielectric may interfere or distort the electric field between the plates, thus weakening the electric field strength and producing an area where microorganisms may pass without experiencing the full field.

The second problem is with imperfections in the manufacturing process at the boundary between the electrodes and the dielectric between them. These imperfections may allow a small amount of water to pass between the electrode and dielectric, thus shielding the water from the full strength of the electric field.

To overcome the size and edge effect difficulties, a concentric cylinder electrode system was designed and constructed as shown in Figure 7.3.

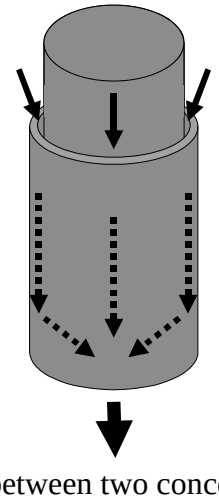


Figure 7.3 Water flow between two concentric cylinder electrodes

Water passes between two stainless steel concentric cylinders which act as electrodes. The gap between cylinders is 1.8mm. The diameter of the inner surface of the outer electrode is 44.6mm. The cylinder overlap length is 10.0mm. The exposure

time or length of time a section of water is between the electrodes is 19 ms at the full flow rate of 10 LPM.

With the concentric cylinder electrode system, edges are removed and all water and suspended microorganisms must pass through the full field. The electric field between the concentric cylinders is slightly non-uniform. The electric field strength at the outer electrode is lower than at the inner electrode due to the electrode curvature. However, the diameter of the cylinder electrodes is large enough to minimise this difference and give an effectively uniform field.

With these design changes the concentric cylinder electrode system was expected to outperform the parallel plate electrode system for the same exposure time and applied electric field.

7.2.6 Flow Switch (FS)

The flow switch used in this device (Gentech flow switch, Farnell 730-804) was relatively sensitive to flow, cheap and readily available.

7.2.7 High Voltage Transformer (HVT)

Larger flow rates mean a corresponding increase in power. Large flow systems that operate at 50 Hz may require distribution level voltages and power systems. A voltage level of 5.5 kV was used because of its relevance to the national power distribution grid. Standard distribution transformers that operate off the 11 kV power lines are commercially available and may suit some large flow rate treatment systems. Often these transformers have two 240V windings that can be configured to give a high voltage of 5.5 kV. For the 10 LPM device however, a custom-made high voltage transformer was sourced from a local manufacturer. This transformer operates on single phase, 240:5500V and is rated at 1 kVA.

7.2.8 Solenoid Valve (SV)

The solenoid valve is mounted externally to the 10LPM device. This makes the design more flexible, as some applications may not require a solenoid valve. A solenoid valve is necessary to prevent the flow of untreated water in event of a fault or depleted resin. However some applications, such as in a recirculating system (air conditioning), may not require the water to be completely shut off. A 240V power socket is provided on the device case to power a solenoid valve if so required.

7.3 Assembled 10 LPM Device

The 10 LPM components were assembled into a standard electrical enclosure. The completed device is shown in Figure 7.4. Figure 7.4(a) shows the external appearance and Figure 7.4(b) shows the location of the internal components.

7.4(a)

7.4(b)

Figure 7.4 Picture of the 10 LPM device (a) external and (b) internal components

7.4 Testing of the Device

The development model of the 10 LPM was installed and tested on the local municipal water supply. The measured power required for operation was about 200W.

Four main problems were encountered in the running of the device. These problems were transformer instability, inrush current, electrode arcing and changes in water conductivity. They affect the stability or robustness of the device. Solutions were needed for each of these problems before the device could be used commercially. The solutions to these problems are discussed in Chapter 8.

Chapter 8

MODIFICATIONS TO THE E10 NEEDED TO SOLVE INSTABILITY PROBLEMS

8.1 E10 Instability Problems

The e10 water purification device is a scaled up version of the original one litre per minute Wateriser but operates at the higher flow rate of 10 litres per minute. Some practical problems were encountered with the device that affect its robustness. These problems were perhaps always present in the 1 LPM Wateriser, but small enough not to noticeably affect its performance. These problems become magnified when the device is scaled up to treat larger flow rates. With the e10 device these problems necessitate a change in design to make the device robust and commercially marketable.

There are four main problems affecting the robustness of the e10. They are transformer instability, inrush current, electrode arcing and changes in water conductivity.

8.1.1 Transformer Residual Magnetism (P1)

Occasionally during the initial testing of the e10, a sudden change or perturbation in the normal operation of the high voltage transformer (HVT) would take place. Sometimes this was due to a high inrush current when the HVT was turned on (See also Section 8.1.2). The inrush current either triggered the overcurrent protection, which turned the transformer off on the next zero crossing (triac controlled), or blew a protection fuse. Another cause of these perturbations was electrical arcing at the electrodes. Arcing had similar effects to the inrush current problem. The primary current was abruptly interrupted either by the overcurrent protection or the fuse.

In either case, after these perturbations the HVT could not be turned back on successfully. A very large inrush current would immediately occur which would then shut the transformer off again. When the transformer was in this state it needed to be turned back on softly (ie. primary voltage increased gradually) to take it back to normal operation. The reason for this was probably due to the residual magnetism of the transformer. The high current drove the transformer into saturation and the sudden interruption of the primary current caused the transformer core to remain highly magnetised.

8.1.2 Transformer Inrush Current (P2)

Often when the HVT was initially switched on, there was a large initial current spike (inrush current). The magnitude of transformer inrush current is dependent on two variables, residual magnetisation and the point in the 50Hz mains waveform where the

switching occurs [Gottlieb 1998]. For the HVT wound for the e10 device, the inrush current magnitude can be as high as 90-100Amps. A large current such as this may trigger the current sensor and current overload alarm, and turn off the device.

8.1.3 Electrode Arcing (P3)

Arcing can sometimes occur between electrode surfaces due to the high electric field. Since the surface area of the electrodes is larger for higher flow rates, this increases the probability of arcing. The electric field strength used for effective biological lysing is 30 kV/cm rms. This field strength is greater than the breakdown strength of air, so any air bubbles within the region of high electric field can electrically breakdown and initiate arcing between the electrodes. Areas in the electrodes that cause the field strength to be concentrated (ie. at entry and exit points where there is a sharp edge) exacerbate the problem. The physical design of the electrode surface is important for this reason. The electrode surface must be smooth to reduce areas of imperfections in the surface which can lead to a concentration of electric field strength or a region where air bubbles may accumulate or stick. All electrode edges (especially entry and exit points) must be slightly rounded to reduce field stresses. It is also good design to misalign the electrode edges so the field stresses at the edge of one electrode does not combine with the edge stresses of the adjacent electrode.

The mechanical design of the air trap and electrode chamber is also important for the reduction of air bubbles at the electrode surface. The air trap volume must be sufficient to slow down the water and enable trapped air to rise to the top. The inner surfaces are smooth to reduce air bubbles being generated or trapped. The electrodes are mounted directly at the bottom of the air trap. The cross sectional area between the electrodes is much smaller than the cross sectional area of the air trap. This causes the water velocity to increase as it flows into the electrodes. The water velocity increase means a corresponding decrease in pressure, according to Bernoulli's equation for incompressible fluids [Sears *et al*. 1982]. Sudden decreases in pressure may cause air to come out of solution, so should be avoided as much as possible. It is not possible, however, to increase the cross-sectional area between the electrodes, or to significantly decrease the cross sectional area of the air trap. The best design is to not reduce the cross sectional area of the water flow until the water reaches the electrodes. This design proved to be the most robust upon practical testing of all designs.

8.1.4 Changes in Water Conductivity (P4)

The e10 water purification system can only operate on low conductivity water. The mixed bed deionising resins reduce the water conductivity so the device will work. The water conductivity required for successful operation is less then one micro-Seimen per centimeter (1 μS/cm). The nuclear grade deionising resins that were used on the e10 device lower Christchurch tap water (100 - 130 μS/cm) to this level. From experience, they operate reasonably well and do not require prior flushing to get the conductivity down to this level. However, when the device has been sitting idle for any length of time, the deionised water in the system can increase in conductivity. The amount of conductivity increase is proportional to the amount of time the device has been sitting unused. This increase in water conductivity can cause large currents to flow immediately the HVT is turned on, either triggering an overcurrent alarm or blowing the fuse on the primary.

When the e10 is sitting idle, deionised water is sitting in the system everywhere downstream of the deionising resin. The volume of water between the deionising resin and electrode chamber has been estimated to be 1.8 litres. This includes water sitting in the hose between the resin and air trap, the air trap, air release valve and electrode chamber. Depending on the flow rate being used (2 - 10 LPM) this would take between 54 and 11 seconds to flush through the system. The flushing time is actually longer, since when the water flow starts, deionised water flows from the resin through the pipe into the air trap where the ions quickly dissociate and gradually reduce the conductivity of the water in the air trap. Thus when the high voltage is turned on, the initial current is high then starts to gradually reduce until a normal current level is reached. For a 2 LPM flow rate this can actually take between 90 and 120 seconds.

Some experiments were undertaken to find the cause of the conductivity increase, with a view to perhaps eliminating the problem. The possible areas of ionic contamination may come from the following sources that are in contact with the water.

- pipe between deionising resin and air trap
- air trap
- air release valve (brass components)
- electrodes (stainless steel)
- downstream of electrodes

By this experimental process of elimination it was found that the bulk of the ionic contamination occurred because of the electrodes themselves. The air trap and pipe components are constructed out of food grade plastics. The air release valve is made of brass, but the majority of this is not in contact with the water itself.

8.2 Solutions to the Instability Problems

The design changes required for solving these four instability problems could be divided into two categories: electrical and mechanical.

8.2.1 Electrical solutions (A)

8.2.1.1 Zero current switching (A1)

Switching the transformer on at the optimum period of the 50Hz voltage waveform can significantly reduce transformer inrush problems [Gottlieb 1998]. For an initial inductive load this occurs at zero current or full voltage. This may be achieved by detecting the zero voltage crossing and adding a 5 msec delay before switching on the HVT triac. A practical circuit was built and incorporated into the e10 device. It was found that this circuit significantly reduced the inrush current. However, occasionally the inrush would still be high enough to trip the overcurrent alarm. This may be due to the residual magnetism of the transformer core. The inrush current can also be load dependent. That is, if the water conductivity between the electrodes is high because the device has been sitting idle, the inrush may be significant.

8.2.1.2 Soft start (A2)

A primitive soft start circuit was built using a small series resistance in series with the primary of the transformer, which limits the initial inrush current. The series resistance is bypassed by a relay that turns on 60 msec after the transformer has turned

on. An aluminium housed, wirewound resistor (22Ω, 100W, RS Components Australia) was used for this purpose. This resistor has a high enough resistance, power and voltage rating to handle brief current surges associated with the HVT turn on. However, it cannot withstand continuous operation, so if the bypass relay fails, the resistor will overheat and also fail.

8.2.1.3 Initial disable alarms period (A3)

The primary current level alarms are disabled for a short period following switch on of the HVT. This is done to prevent the alarms from turning off the e10 until after the inrush current has subsided. However, with the alarms disabled there is no protection in the event of arcing or other sudden current surge. The alarms can thus only be disabled for a short period (about 60msec). This does not solve the problem of current surges caused by increases in the water conductivity, as these can last up to 120 seconds.

8.2.1.4 Series resistance (A4)

A permanent series resistance can be added to the primary of the HVT. This will limit the primary current surge. The advantage of using a series resistance is it makes the system electrically robust. The primary current is limited during turn on, but also when arcing occurs at the electrodes. This will reduce electrical stresses on the fuse, triac and other in-line devices. The resistance wastes power, but this is a small price to pay since the operating power of the whole device is already low (the resistance would add only 10% onto the overall power consumed by the device under normal operating conditions). The main disadvantage is obtaining a power resistor than can handle the peak power ratings.

From observations, the nominal power level for the e10 is around 200W under normal operating conditions. This may vary slightly depending on the deionising resin and source water quality. This equates to a current of 0.83A. A 20Ω series resistance would dissipate 17W of power under normal operation. Shorting the secondary of the HVT would cause the worst case power surge. The full 240V AC may appear across the series resistance and the current may peak at 12A. The power dissipated by the resistor at this current level is 2.9kW. Even though the circuit will detect this high current and turn off the HVT, this could take anywhere from 5 to 100 msec. A power surge of this magnitude can damage many types of resistors.

Ceramic power resistors can be obtained from Kanthal Australia Pty. Limited. These non-inductive resistors can absorb high amounts of energy and are able to handle the e10 power surges without damage. A 20 Ohm, 150 W ceramic resistor is rated for 4 kV and 1.6 kJ. This resistor is 1 inch in diameter and six inches long. It costs A$124.90. The size of the resistor would create mounting difficulties in the e10 enclosure. When continually dissipating 20W, a fan cooling system would be necessary to shift the heat to the outside of the enclosure.

Kanthal Australia also stocks power resistors that are water-cooled. The water coolant flows inside the resistor. This may enable the resistor to be part of the plumbing and keep it working cool, negating the need for fan cooling. The problem with this resistor is its price (A$538.40). There is also uncertainty about the resistor water path being made of food grade material. For these reasons the permanent series resistance was not implemented in the e10.

8.2.2 Mechanical solutions (B)

8.2.2.1 Elimination of metal parts (B1)

The e10 has been designed to minimise water exposure to metallic parts that could cause ionic contamination. All materials have been selected to be food grade. The metallic parts that may be in contact with the water are the electrodes (stainless steel) and air release valve (brass). There is no way to avoid having the electrodes in contact with the water, as this is the basis of the disinfection capabilities of the device. The electrodes however may be made of a different metal type that is more passive (eg. titanium).

No plastic air release valves of the appropriate size could be found. If the ARV did cause a problem, the brass may be coated with a conformal plastic coating to prevent contact with the water. However, experiments have shown that the ARV does not significantly contribute to ionic contamination of the deionised water.

8.2.2.2 Diverter valve (B2)

A second solenoid valve is installed on the downstream end of the e10 device. This diverter valve is connected to a drain. This enables the device to first drain the deionised water that has been sitting in the system (and may be contaminated) before the HVT is turned on. This is a robust solution to any changes in water conductivity, and is a standard method of stabilising an industrial deionising resin before use. The disadvantages are the waste of good quality water and the extra electronic logic required to control the valves.

8.2.3 Summary of Methods

A summary of the different solutions for the e10 instability problems is shown in Table 8.1.

Solution	Problem				Notes
	P1	P2	P3	P4	
A1. Zero current switching	❍	◗	◗	❍	Only a partial fix
A2. Soft start	●	●	◗	❍	Cost minimal
A3. Initial disabling of alarms	❍	◗	❍	❍	
A4. Series resistance	●	●	◗	◗	Cost A$125 - A$480
B1. Elimination of metal parts	❍	❍	◗	◗	Cannot eliminate electrodes
B2. Diverter valve	❍	❍	◗	●	Cost NZ$250 + extra circuitry

where ❍= no effect; ◗ = partial effect; ● = full effect.

Table 8.1 Comparison of solutions for the e10 instability problems

Considering the advantages and disadvantages of these design solutions, the best method is to use a combination of soft start (A2) and diverter valve (B2). The diverter valve is reasonably expensive (NZ$250) due to the addition of an extra solenoid valve, however it offers significant advantages in terms of reliability and robustness. These two qualities are of vital importance if this device is to be marketed and distributed to potential customers.

The only problem that is not entirely fixed by the implementation of these design changes is that of electrode arcing (P3). The arcing problem can only be effectively reduced with good electrode design.

The addition of a diverter valve requires changes to the control electronics of the device, adding to its complexity. For this reason a completely new control circuit was designed that uses a microcontroller. The microcontroller replaces much of the discrete logic that controlled the initial prototype. The use of a microcontroller also gives more control and "intelligence" to the device. Since one disadvantage of using the diverter valve is the waste of water, water may be conserved by the microcontroller monitoring the water conductivity and only diverting water to waste if the conductivity is above a certain level. The microcontroller is described in more detail in Chapter 9.

Chapter 9

MICROCONTROLLER

9.1 Introduction

The initial model of the 10 LPM treatment device (e10) used discrete logic for the electronic control. The incorporation of a microcontroller is advantageous, especially for the industrial devices. A microcontroller replaces much of the discrete logic, thereby lowering the cost of board manufacture. It gives the device more flexibility - minor changes in the operation of the device may be made in software without any hardware changes. This will enable a generic hardware design for e10 devices with different modes of operation.

9.2 Microcontroller Functions

9.2.1 Accurate real time monitoring of the high voltage transformer primary current.

The output from a current transformer is fed into the A/D converter of the microcontroller. When the current rises above the two alarm levels (arc and resin) for the specified time, the alarms can be triggered. This function is similar to that of the analogue control circuit, except that current alarm levels may be set and changed in software rather than manual adjustment of analogue components.

9.2.2 Automatic reset after arc or resin alarms.

In the event of an alarm, the e10 can be made to reset and restart itself. This minimises the need for manual resetting and human intervention. If an arc alarm occurs, the microcontroller can shut down the operation of the device, and begin a self-restart process after a given amount of time. The device can be made to self restart a number of times. If the alarm triggers a number of times in a given time frame, then the device will shut down and wait for manual intervention.

9.2.3 Option of intelligent flushing or drain sequence.

From Chapter 8, water drains to waste for a set period (10 seconds) to stabilise conductivity before switching on the high voltage. This is required to prevent power surges at start-up that can lead to false alarms and instability. These power surges are due to an increase of water conductivity between the electrodes and in the air trap. The conductivity increases occur when the device has been sitting idle. The longer the periods between use, the more of a problem the power surges become.

An intelligent flushing or drain sequence procedure can be set in place by use of the microcontroller. When the high voltage is initially turned on, the primary current is

averaged over the first 60 ms. If the average current is higher than a set threshold, the high voltage is disabled and the drain sequence continues. The microcontroller uses the high voltage to keep 'polling' the electrodes every two seconds. In this way the device only switches fully on when the conductivity is low (<1 μS/cm) and the high voltage can operate safely. The minimum amount of water is wasted as the drain sequence only operates when required. The water out of the device is also guaranteed to be the highest purity (<1 μS/cm).

9.2.4 Monitoring of alarm history.

The microcontroller stores the following information:

1) Number of Arc alarms
2) Number of Resin alarms
3) Number of Drain sequences
4) Number of Controller resets
5) Number of Manual resets

The data obtained will aid a field technician to ascertain the fault of the device or its environment. The data can be downloaded to a computer via a serial (RS232) cable.

9.2.5 Serial communication port.

A serial connection is provided so an external device may interrogate the microcontroller. A field technician may download alarm history to a laptop PC. This also allows provision for some communication device to be installed so the e10 may be interrogated remotely. This may allow for a centralised service centre to maintain a number of remotely installed devices.

9.3 Modes of Operation

The different modes of operation for the e10 are listed in Table 1. This table shows the hardware and software requirements for each mode of operation.

	Storage tank		**Point-of-use (POU)**		**Recirculation**
	Sensors	Mechanical	Normal	Tests	
Hardware					
Level sensors	●				
Ball-cock		●			
Flow switch		●	●	●	●
Water solenoid	●	●	●		
Drain solenoid	●	●	●		
Software					
Sensor control	●				
Flow switch control		●	●	●	●
Resin alarm	●	●	●		●
Arc alarm	●	●	●		●
Auto-reset	●	●	●		●
Solenoid control	●	●	●		●

Table 1 Hardware and software requirements for different modes of operation

There are five different modes of operation, but only three different software packages. The recirculation mode is the same as the normal point-of-use mode except that the water and drain solenoids are not physically installed.

9.3.1 Storage tank

9.3.1.1 Level sensors

One or two float switches or other level sensors are installed in a storage tank. The level sensor(s) control the operation of the e10. With one level sensor the e10 switches on/off more often due to the small amount of hysteresis in the sensor. With two sensors the on/off hysteresis may be made much larger (eg. the e10 turns on when the tank water level has dropped to below halfway), and the e10 operates for longer periods with less turn off and on.

9.3.1.2 Mechanical valve

A mechanical cut-off valve may control the filling of a tank. This may be some sort of float or ball-cock valve. In this case, the flow switch would control the operation of the e10. When the tank is full, the valve closes and the flow stops, switching off the high voltage.

One problem with this mode of operation is that normal ball-cock valves do not have a defined cut-off point. As the tank fills the valve begins to close and the inlet flow rate drops slowly. Thus the flow rate may drop below the sensing level of the flow switch, and the high voltage would turn off before the tank is completely full. Double action ball-cock valves are available that give a sharp flow cut-off. These types of valves are therefore required for this mode of operation.

9.3.2 Point of use (POU)

The point-of-use mode of operation is used for applications that require the water to be purified immediately before use. There is no storage of the purified water. This mode of operation would be the most common application for this technology. The flow switch controls the operation of the high voltage.

The normal mode of POU operation would have a control solenoid valve installed. The valve shuts off the flow of water if there is a fault (arc/resin alarm, or loss of power to the device), ensuring untreated water does not pass through. However, some applications may require an uninterruptible water supply and thus no solenoid would be installed. Similarly there is an option of a drain sequence or no drain sequence. When testing the effectiveness of the e10 electrode system on bacteria for example, there is no solenoid or drain sequence installed, and the alarms are disabled. The high voltage is monitored by meter and controlled manually by mains power to the device.

9.3.3 Recirculation

The e10 may be used in recirculation systems such as air conditioning systems. These applications do not require 'failsafe' operation; therefore there would be no water solenoid valve required. The drain sequence would still be operational, but no drain solenoid installed (this enables the high voltage to be stabilised when the e10 is switched on without any waste of water). The flow switch controls the operation of the high voltage.

9.4 Discussion

The use of a microcontroller to control the operation of the 10 LPM purification device has many advantages. It provides more flexibility in the operation of the device. The device may be used in different operational modes with the microcontroller programmed differently for each. The hardware may remain generic for ease of manufacture.

The microcontroller makes the device smarter. Alarm or fault situations may be handled easily and the device can automatically clear itself and recommence operation. When a drain valve is installed, the microcontroller can continuously monitor when it is required, thereby minimising the amount of wasted water.

The microcontroller stores information about the performance of the device. It has a serial port and can communicate with a computer, either at location or remotely from a central location.

Chapter 10

A 33 LPM TREATMENT DEVICE

A 33 LPM device was constructed using a similar design to the 10 LPM air trap and electrode chamber. The flow rate of this new electrode chamber matches the maximum specifications of a larger deionising resin column (Ionmiser30, US Filter NZ, Auckland). This is the maximum sized standard resin column that is still portable. Larger resin columns may be constructed on-site to suit the size of any application. It is envisaged that three of these resin columns with associated treatment devices may be installed in parallel to give a treatment capacity of 100 LPM.

10.1 System Design

The 33 LPM device is very similar in design to the 10 LPM device, as described in Chapter 7. The air trap and electrode chamber system is a scaled up version of the 10 LPM components. The high voltage transformer has a higher power rating (3.3 kVA) than that of the other devices.

10.1.1 Air Release Valve (ARV)

The volume inside the air trap is much larger than that for the 1 LPM and 10 LPM devices. A large volume of air may need to be released quickly. Therefore the mechanical air release valve used by the 1 LPM and 10 LPM devices was not adequate. A larger valve would need to be used, but could not be immediately sourced. For this reason a solenoid valve (240V, normally closed) was used to control the release of air. A level sensor was mounted on the side of the air trap and this operated the air release valve.

10.1.2 Air Trap (AT)

The 33 LPM air trap was identical in construction to the 10 LPM air trap, except it used larger pipe components (150mm Class C uPVC pressure pipe). End caps were attached to either end of a 0.65m length of pipe. Holes were drilled and tapped for inlet and outlet, air release valve and level sensor.

10.1.3 Electrode Chamber (EC)

The 33 LPM electrodes were made of stainless steel concentric cylinders. The design was very similar to that for the 10 LPM device. The assembled electrode chamber is shown in Figure 10.1.

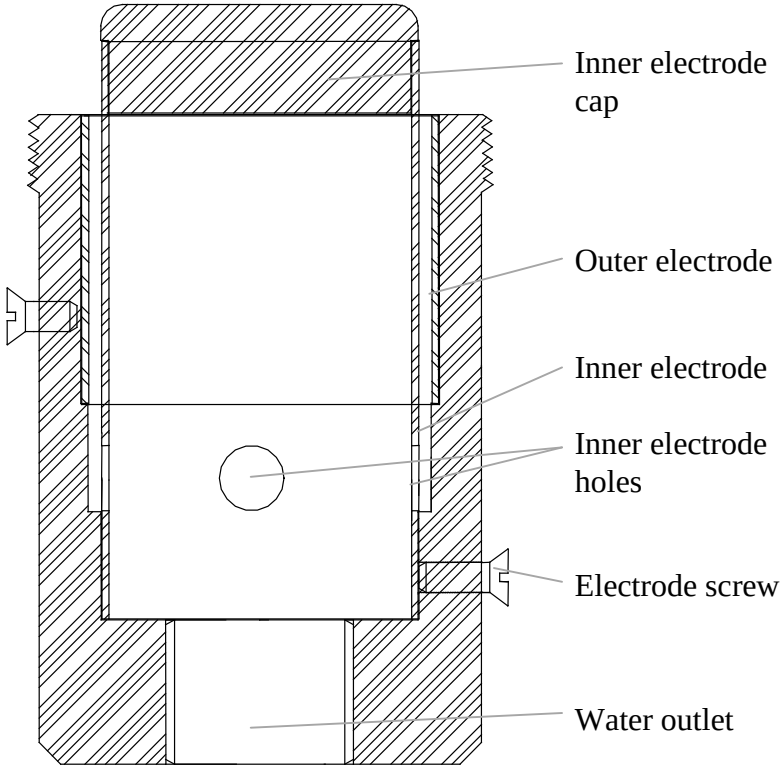


Figure 10.1 Assembled electrode chamber.

The electrode chamber is mounted at the base of the air trap and screws in for easy removal. The gap between the inner and outer electrodes is 0.18 cm. The diameter of the inner surface of the outer electrode was 4.445cm. The electrode overlap length was 4.0cm. A tight fitting cap was placed on the inner electrode to prevent the entry of water. The main body and inner electrode cap were machined out of high density polyethylene. Both cylindrical electrodes were a press-fit into the main body. The inner electrode had four 0.8cm holes drilled at a position about 1cm below the overlap of the two electrodes. These holes allowed for the water to pass through to the outlet. Electrical contact was made with the two electrodes by the insertion of two stainless steel grub screws.

10.2 Assembled device

The 33 LPM air trap and electrode chamber and attached components were assembled. The assembled device was mounted vertically on a steel frame. The assembled device is shown in Figure 10.2.

A solenoid valve (240V, normally closed) was mounted at the inlet to the air trap. This valve controlled the flow of water. The level sensor was mounted at least 0.1m above the water inlet and at right angles to the inlet flow. This is to minimise the effects of turbulence and water flow on the level sensor. The normal water level is just above the level sensor. When the water level drops below this point, the air release valve turns on to release the air. Pressure within the air trap forces the air out until the water level again rises above the sensor. When the air release valve turns off, the water level still rises slightly due to the compression of the remaining air. This effect leads to a slight hysteresis and enables the use of a single sensor.

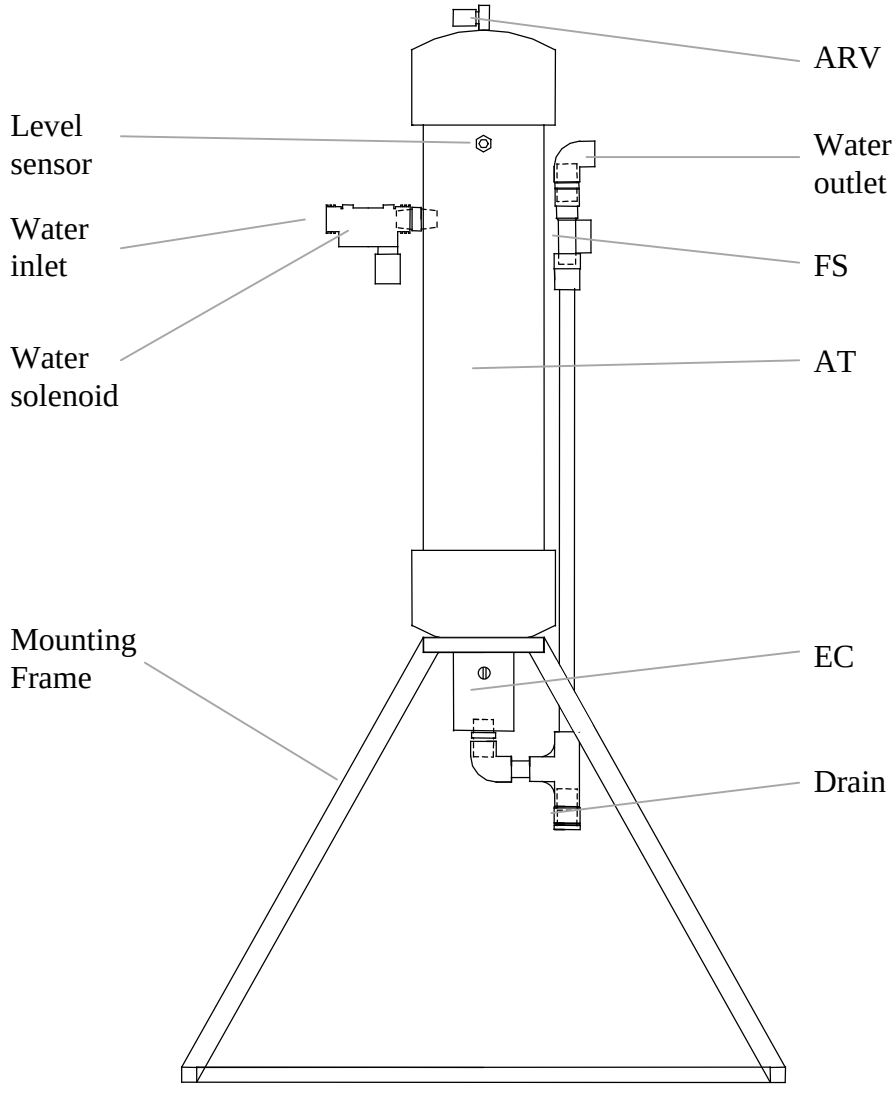


Figure 10.2 Assembled 33 LPM device showing air release valve (ARV), air trap (AT), flow switch (FS) and electrode chamber (EC).

The tee section in the outlet PVC plumbing allowed for the system to be drained of water. A flow switch was mounted vertically next to the air trap. The flow switch was the same type as that used in the 10 LPM device.

10.3 Testing of the 33 LPM Device

The 33 LPM device was constructed in order to test the electrical and water flow characteristics and to test the effects on bacteria. For this reason the device was not fully assembled (i.e. case, control electronics, high voltage transformer) to a point where it would be able to be used commercially. Instead, biological and electrical testing was done in a high voltage laboratory using an adjustable high voltage, high current supply. The voltage levels were then adjusted and controlled manually to the appropriate levels.

The larger surface area of the electrodes causes them to be less robust. Probability of an air bubble or electric field stress within the electrodes is increased. Therefore,

electrodes with larger surface area are more prone to electrical breakdown and damage at the electrode surface.

The power rating of the high voltage transformer is significantly greater than the 10 LPM device. Thus, when an electrical breakdown occurs, there is less impedance to limit the flow of power. Breakdowns thus contain more energy and can cause significantly more damage, both in terms of electrode surface damage, and in electrical current spike in the primary circuit. The nominal operating power for the 33 LPM device was around 600W once the deionising resin had stabilised.

The next step after successful testing would be to implement the design changes discussed for the 10 LPM device, as described in Chapters 8 & 9. These design changes and controller would need to be implemented in order for the device to perform robustly.

The 33 LPM device was also tested on bacteria. These results are discussed in Chapter 11.

Chapter 11

PERFORMANCE OF LARGE FLOW DEVICES ON BACTERIA

This chapter investigates the performance of the large flow rate devices (10 LPM and 33 LPM) on bacteria. It is shown that the effect of these electrode systems is not as high as the slow flow rate Wateriser. Possible reasons for this low performance are suggested and the effects of these are explored.

11.1 Test Methods

The experimental procedure used in this testing was similar to that used for the initial 1 LPM device (Chapter 4). In order to test the effectiveness of the high voltage field alone, the deionising resin was not used as part of the experimental apparatus. The bacteria *Serratia marcescens* was cultured in 10ml nutrient broth and incubated at 30°C overnight. The bacteria was then harvested in the early stationary growth phase, washed and resuspended in deionised water. The inoculated water was injected into the system immediately after the deionising resin using compressed air (Figure 11.1). The inoculated solution was injected at a constant rate over a period of two minutes. The high voltage was initially switched on and a sample was taken after one minute of injection to allow for bacteria levels to mix and stabilise inside the air trap. Immediately after the sample was taken, the high voltage was disabled and then a control (untreated) sample taken.

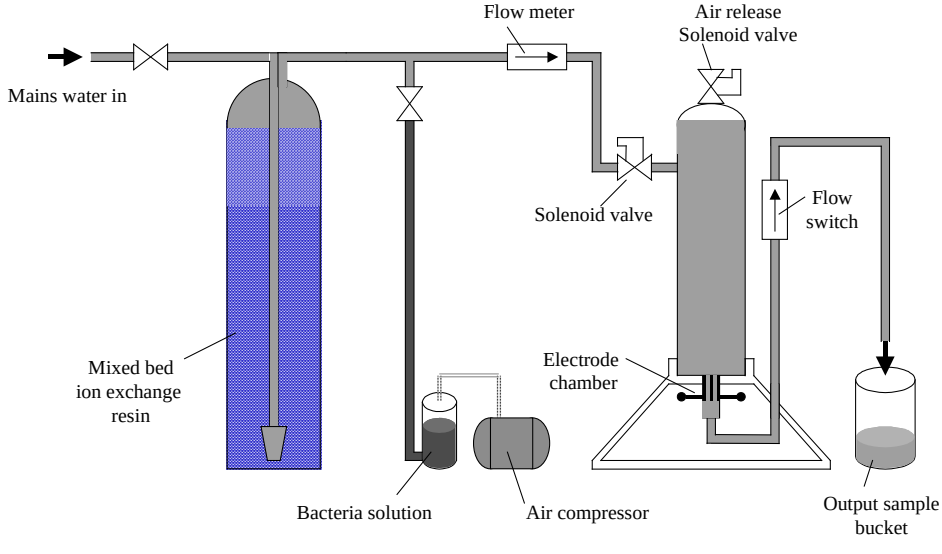


Figure 11.1 Experimental apparatus for testing the industrial devices

The treated and untreated solutions were then serially diluted and plated onto nutrient agar. Each plate was done in duplicate and the average result reported. The agar plates were incubated at 30°C for 24 hours and the resulting number of colony forming units (CFU) counted.

Three different electrodes were used in the 33 LPM device. The first two electrodes were physically identical (to check any variations in performance due to manufacturing tolerances), and the third was constructed with an aspect ratio of 1 (to check variations in performance due to different aspect ratios, see Section 11.2.1). The 10 LPM electrode used was similar in construction to the first two 33 LPM electrodes, but shorter in length.

11.2 Test Results

Both the 10 LPM and 33 LPM water purification devices were tested on water inoculated with *Serratia marcescens*. The survival ratio of this bacteria passing through the device is shown in Figure 11.2 where it is compared with the results from the 1 LPM domestic device.

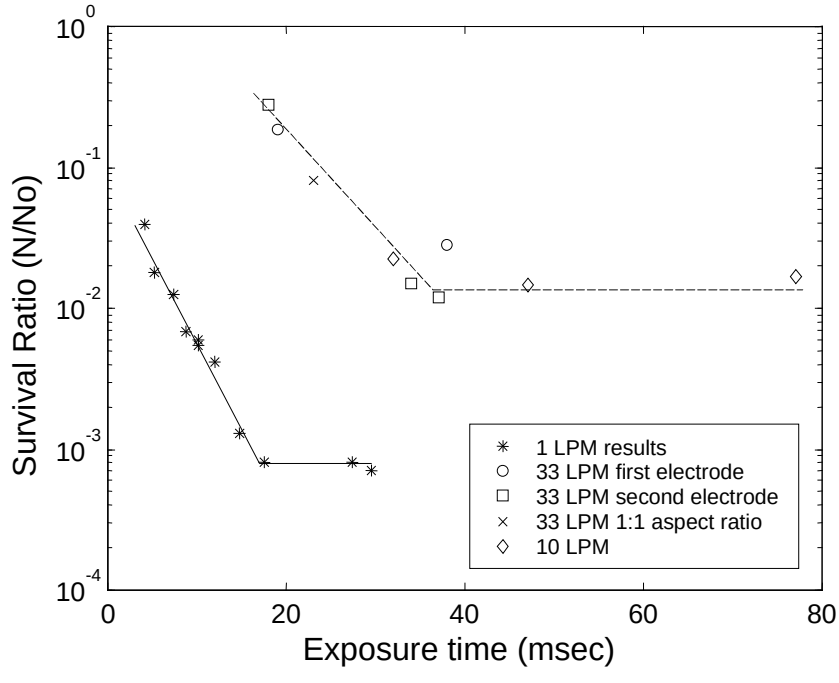


Figure 11.2 Survival ratio of *S. marcescens* from devices with different flows

All three 33 LPM electrodes and the 10 LPM electrode gave a similar performance. It can also be seen that the survival ratio of *S. marcescens* is significantly higher for both industrial devices (10 & 33 LPM), even at higher application times, than that for the domestic 1 LPM device. At the maximum flow rate of the 33 LPM device (an exposure time of 17 msec) the survival ratio is only 0.3 (70% lysing). The 1 LPM domestic device at maximum flow rate and identical exposure time gave a survival ratio of 0.001 (99.9% lysing).

Figure 11.3 shows the 33 LPM results plotted against flow rate. It shows that over the range of rated flow for the 33 LPM device (12-33 LPM) the survival ratio varies

logarithmically from 0.01 to 0.5. This flow rate range corresponds to an exposure time range of 35 msec - 17 msec. For equivalent exposure times in the 1 LPM device the survival ratio was constant at 0.001 (Figure 11.2). For the industrial devices, the survival ratio becomes constant with an exposure time of greater than 35 msec, double that of the 1 LPM device. Also the survival ratio threshold for the industrial devices is more than one log higher than that of the 1 LPM device.

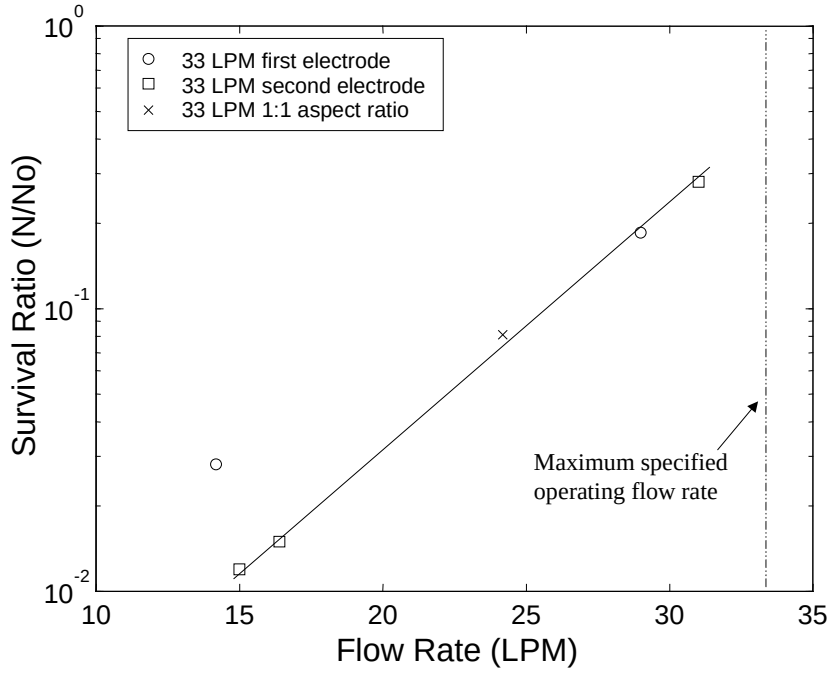


Figure 11.3 Survival ratio of *S. marcescens* as a function of flow rate for the 33LPM device

Based on these results from testing on *S. marcescens*, the effectiveness of the 33 LPM device at the maximum flow rate is too low for a commercial treatment system. Even if the maximum specified flow rate were lowered to half of this value (17 LPM) the effectiveness may still be too low. The effectiveness of this device therefore needs to be improved before commercial development can proceed.

In order to solve these performance problems, the reasons behind them must first be understood. A list of physical and experimental differences between the domestic and industrial device experiments is shown in Table 11.1. Some of the differences listed in this table may contribute to the poor performance on *S. marcescens*.

	1 LPM	***10 & 33 LPM***
Physical parameters		
Flow rate (LPM)	1	10 / 33
Electrode shape	Parallel plate	Concentric cylinder
Aspect ratio (width/length)	1	0.54
Electrode gap (cm)	0.2	0.18
Applied voltage (kV rms)	6	5.5
Biological Testing		
Initial bacteria concentration (CFU/ml)	100,000 - 300,000	15,000 - 120,000

Table 11.1 Comparison between 1 LPM, 10 LPM and first two 33 LPM electrodes

Some of the major differences between the domestic and industrial devices that could be contributing to the low performance on *S. marcescens* are the aspect ratio, electrode gap, initial bacteria concentration during testing, and the possibility that bacteria may be clumping. Each of these differences is explored in the following sections.

11.2.1 Aspect Ratio

The aspect ratio is defined as the water channel width between the electrodes divided by the length. In the 1 LPM parallel plate electrodes, the electrode width equals that of the electrode length (ie. 1 cm). The aspect ratio is thus equal to 1. The 33 LPM electrodes have an aspect ratio of 0.54. The range of available stainless steel pipe sizes dictated the diameter of the cylindrical electrodes and the length was calculated from the maximum flow rate required. Edge effects occur at the water entry and exit points. The electric field strength at these edges is reduced between the electrodes at a small distance from the edge. It is possible this reduction in electric field slightly lowers the effective electrode area. For electrodes with low aspect ratio, the electrode width is increased in comparison to the length, thereby magnifying the effects of the edges.

However, if the smaller aspect ratio caused a decrease in effective application time, then this is more than offset by the tests in Figure 11.2 being undertaken at higher exposure times. That is, the exposure times for the 10 LPM and 33 LPM experiments were significantly higher than that for the 1 LPM experiments. This should more than compensate for any decrease in exposure time caused by the low aspect ratio.

To verify that the difference in aspect ratio is not contributing to this difference in results, a new 33 LPM electrode with an aspect ratio equal to 1 was designed and constructed. The average circumference and electrode overlap length was 6.0 cm. The electrode gap was identical to the other 33 LPM electrodes (0.18 cm). The aspect ratio is the same as that for the 1 LPM electrodes.

Testing was undertaken on *S. marcescens* under similar conditions to previous experiments. The survival rate of the bacteria closely matched that of the previous electrode (Figure 11.2). This confirms that the aspect ratio differences between the 1 LPM and 33 LPM electrodes is not a likely contributing factor in the survival ratio differences.

11.2.2 Electrode Gap

Due to the difficulties in constructing concentric cylinder electrodes with differing or adjustable gaps, a smaller parallel plate electrode system was constructed for this purpose. It was thought that variations in electrode gap on a small scale may be representative of gap variations in a larger concentric system. If differences in the performance on bacteria were detected for different gaps on a small system, this would indicate the same could be true of a larger system.

A small, parallel plate electrode system with an adjustable electrode gap was constructed. The electrodes were made of stainless steel and measured 0.5 x 0.5cm. The electrode gap was adjustable between 0.065 and 0.20 cm. Depending on the actual gap, the maximum flow rate these electrodes were designed for (at an exposure time of 17 msec) was around 0.1 LPM.

The 0.1 LPM electrodes were tested on *S. marcescens*. Four different gaps were chosen, 0.065, 0.089, 0.113 and 0.200 cm. As the gap was varied, the applied voltage

was also varied to maintain the electric field constant for all experiments. The applied electric field was 30 kV/cm rms, 50 Hz. The experimental results are shown in Figure 11.4.

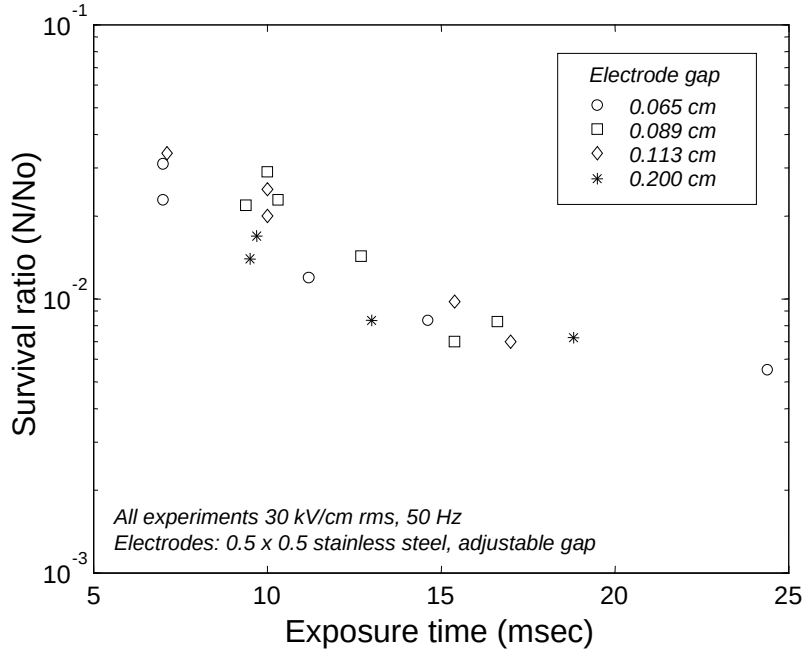


Fig 11.4 Effects of electrode gap on the survival ratio of *S. marcescens*

The points for all experiments cluster about a common trend line regardless of gap width. Therefore it would seem that variations in electrode gap do not have any significant effect on the survival ratio of *S. marcescens*.

Also, there is a logarithmic decrease in survival ratio with increasing exposure time until it flattens out at about 17 msec. This is very similar to the 1 LPM results. However, the survival ratio limit in this case is about 0.006, which is higher than the 0.001 limit shown by the 1 LPM electrodes (Figure 11.2).

11.2.3 Initial Bacteria Concentration

For experiments with high bacteria levels, an increase in survival ratio may occur. This is due to a 'shielding' effect. That is, some bacteria shield other bacteria from the effects of the electric field. This can occur when bacteria are in close proximity (touching) or when bacteria are clumped together.

However, as can be seen from Table 11.1, the bacteria concentration in the 33 LPM experiments was actually lower than that for the 1 LPM experiments. This should make the bacteria easier to lyse, due to a lower shielding effect. Bacteria concentration can therefore be dismissed as a factor influencing the lower performance of the higher flow devices.

The effect of initial cell concentration on the survival ratio has been examined by a few authors. Hulsheger *et al*. (1981) found a slight increase in the survival ratio appears when initial bacterial cell concentrations are lowered below 10^5 per ml. This is an opposite effect to what has been postulated in this thesis. The reason for this behaviour was not discussed by the authors, however it has been suggested that at higher concentrations bacteria clumping may occur due to electrostatic forces. This bacteria clump would experience a higher electric field due to the increased dimensions. Thus, a

higher transmembrane potential develops across the cluster of cells than across an individual cell. For this reason, cells can be killed more easily when their number density is high [Jayaram 1992b]. The opposite effect was obtained by Zhang *et al*. (1994b). They found a greater reduction in numbers occurred when the initial cell concentrations were low. They tested on *S. cerevisiae* and found that after a 4-log reduction, the numbers could not be reduced any further. They suggest this may be due to some cluster protection mechanism. The organisms electrostatically cluster or clump together, whereby some cells are protected from the electric field. Mazurek *et al*. (1995) found the initial concentration of bacteria did not have any significant effect on the survival ratio.

11.2.4 Bacteria Clumping

When bacteria exist in clumps, adhering together in large numbers, bacteria in the centre may be shielded from the full effects of the electric field and thus be protected from its lethal effects. In general, bacteria have a slight negative charge, which can often lead to electrostatic attraction.

An experiment was undertaken where the initial bacteria solution was first passed through a 1.6 μm filter to filter out and remove large objects such as bacteria clumps. The resulting filtered solution was then fed into the electrode system to measure the change in survival ratio.

Filtering the solution removed a large number of the actual bacteria as well as any clumps. However, the bacteria numbers were high enough for a clear scientific result. The survival ratio was not significantly different between filtered and unfiltered solutions, as shown in Figure 11.5. Indeed, the survival ratio of the filtered solutions is higher than the unfiltered ones. This agrees with the findings of Hulsheger *et al.* (1981) that low bacterial concentrations tend to have a higher survival ratio, as discussed in Section 3.3. The result tends to suggest that either no bacteria clumps are normally present in the inoculated solution or that bacteria clumps do not significantly contribute to differences in survival ratio when using this method of disinfection. Therefore it is unlikely that clumping would be causing the survival ratio differences between the 1 LPM and 33 LPM devices.

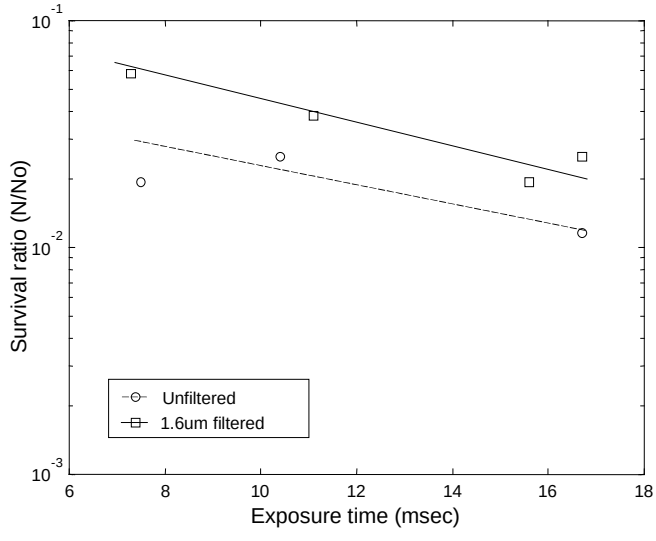
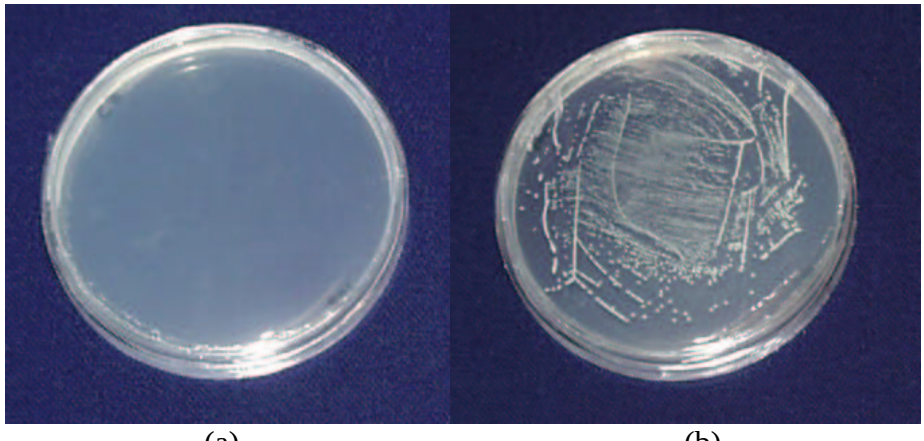


Figure 11.5 Comparison between filtered and unfiltered source solutions of *S. marcescens* exposed to 30 kV/cm rms, 50 Hz.

11.3 Biological Contamination of the Mixed-Bed Deionising Resin

Testing the microbial efficiency of the 33 LPM water purification device involved inoculating a large volume of water with the organism under test. Since such a large volume is required due to the high flow rates, it was impractical to sterilise the water before the experiment. It was assumed that any inherent contamination would be of sufficiently low level when compared to the inoculation levels, and would therefore be insignificant in the final results. Most of the experiments involved Serratia marcescens, which is also pigmented red and may be easily distinguished from background contamination.

These experiments used nutrient agar plates, which are not specific for a bacteria type but will support growth for a wide range of organism types. As a result, in many of the resulting plates a small number of contamination colonies were observed. This did not significantly affect the results since the contamination level was low and the inoculated bacteria colonies could be readily distinguished. However, occasionally an overwhelming number of contamination colonies were observed on an incubated plate. This contamination could occur either on a treated sample or untreated control, but was more prevalent on treated samples.



(a) (b)

Figure 11.6 Nutrient agar plates with 100µl of an identical sample after incubation at 37°C for 24 hours. The sample had been treated by a 50 Hz high voltage of 30 kV/cm with an application time of 34 msec. Plate (a) shows no microbial growth. Plate (b) shows a very large number of colonies.

An example of this type of contamination can be seen in Figure 11.6. Plate (a) has no growth of colonies whereas plate (b) has an overwhelming number of identical colonies. All colonies on plate (b) look to be similar suggesting the same organism throughout. Both plate (a) and plate (b) have been taken from the same sample solution. This sample had been through the water purification unit with the high voltage operating. Normal biological protocols were followed in the assay of this sample. The sample was collected in a sterile 20ml universal container and shaken by hand for 30 seconds to mix bacteria in the solution and give a uniform concentration. The bacteria plating was performed in a biological safety cabinet and standard sterilisation procedures followed (70% ethanol and flaming of glass spreader). The resulting differences in plates (a) and (b) clearly show that the bacteria in solution were not mixed thoroughly. It is likely that the bacteria in plate (b) existed in the water sample in a large 'clump'. This clump of bacteria was not broken up or dislodged by shaking the

solution by hand. The large number of bacteria in the clump may have survived the electric field because of shielding effects. That is, the bacteria on the inside of the clump are protected or shielded in some way from the effects of the electric field. When the resulting bacteria clump were spread onto the agar plate, the bacteria were physically separated, and a large number of colonies grew after incubation.

This poses the question of where did the clump originate? In his review paper, Fleming reports many findings of bacterial growth in ion exchange resin beds [Fleming 1987]. Bacteria in resin beds find enough organic material to live and breed. Since the resin adsorbs matter from the water passing through, it can be a ready source of the nutrients bacteria need to grow and propagate. Another possible source of carbon is the material leached out of the resin itself.

It is thought that the contamination observed has originated from within the mixed bed deionising resin. This deionising resin has been in intermittent operation over the past 24 months, and has been sitting idle for extended periods. This intermittent operation would encourage the growth of bacteria within the resin. It is postulated that the contamination bacteria have formed a biofilm within the resin. Sudden changes in water flow (turning off and on) may dislodge clumps of these bacteria from the biofilm. These clumps pass through the outlet of the resin bed, and through the electrodes where large numbers of bacteria are shielded from the high voltage disinfection.

11.4 Conclusions

The application of high voltage to industrial sized water treatment systems has been examined following the successful development of a 1 LPM device for domestic drinking water. Two prototype devices have been constructed and tested that operate on maximum flow rates of 10 and 33 LPM respectively.

The effectiveness of both industrial sized high voltage water treatment systems in lysing the bacteria *S. marcescens* has been shown to be significantly less than that shown by the 1 LPM device.

Possible reasons for this difference in performance between the domestic and industrial systems were conjectured to be differences in the aspect ratio, electrode gap, or testing methods. Each of these reasons was explored and it was determined that it is unlikely that any of these differences cause the decrease in performance. There seems to be no immediate explanation for the lower performance of the industrial sized systems.

During bacteria testing of the 33 LPM device, contaminating bacteria were found on some of the plates after incubation. This contamination was present on plates of samples that had been electrically treated. The contamination often appeared on one plate, while the duplicate plate was clear, suggesting a group or clump of bacteria. It is likely that this clump of contaminating bacteria is originating from the deionising resin.

The lower performance of the industrial systems may prove significant in the up-scaling and further development of this technology.

Chapter 12

APPLICATION OF A HIGH VOLTAGE SMPS IN WATER DISINFECTION

12.1 Introduction

The process of cell inactivation due to pulsed electric field (PEF) treatment depends upon both applied field strength and exposure time. Increasing the electric field strength has the greatest effect in increasing the amount of inactivation. Increasing the exposure time also increases the amount of inactivation, but to a lesser degree. The most effective voltage waveform would thus be DC. However, DC waveforms pose electrolysis problems (the electrode surface quickly degrades), present greater risk in terms of operator safety and system insulation, and are less robust to the generation of air bubbles and arcing between electrode surfaces. Higher frequency waveforms reduce most of these problems, but have less effect on the reduction of bacteria.

The applied voltage waveform used by the treatment devices described earlier in this report was 50 Hz AC. 50 Hz was used because of its ready availability (national power distribution frequency) and because of its ability to reduce electrolysis problems.

These 50Hz devices use an optimum exposure time of 17ms. The reason for the relatively large exposure time is due to the 50 Hz sinusoidal waveform. There are regions in any sinusoidal waveform where the voltage is low (either side of a zero crossing). The field strengths in these regions may be below the critical strength (E_c) needed for membrane disruption. Thus the exposure time must be large enough to ensure all water has been exposed to a high voltage peak.

This section explores the advantages that can be obtained by using a switch-mode power supply (SMPS) to replace the 50 Hz high voltage supply. A high frequency SMPS may further reduce electrolysis while generating an alternating high voltage DC. Low electric field regions in the waveform are dramatically reduced. The SMPS thus retains some of the advantages of a DC supply, without its disadvantages.

12.2 Apparatus

A high voltage SMPS was constructed that provides a maximum rms value of 3 kV chopped DC at 17 kHz (Figure 12.1). A variac was used to provide a single phase 50Hz variable input (0-230V). The SMPS rectifies the input voltage to provide a 0-320V DC bus. Two IGBT switches (BUK854-800A) operating in a half-bridge configuration chop the DC bus. The resulting 17 kHz chopped waveform is fed through a step-up pulse transformer to increase its magnitude by a factor of 9.4. The 0-3 kV chopped waveform is applied directly to the electrode chamber. The power rating of the SMPS is 400VA.

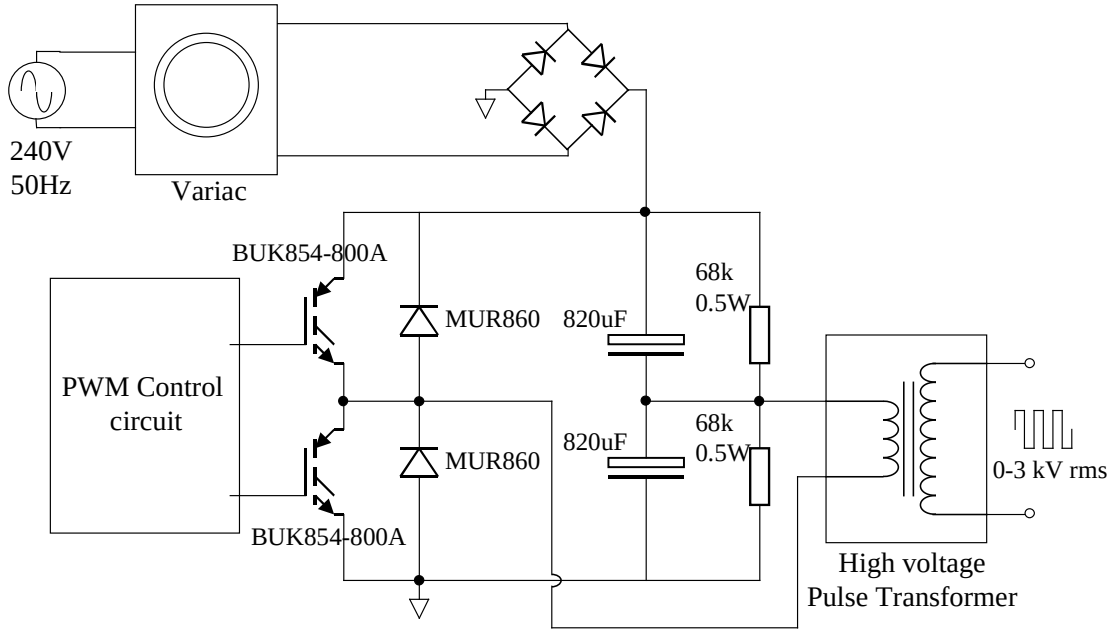


Figure 12.1 High voltage Switch-Mode Power Supply

An electrode chamber was constructed using 5mm stainless steel (grade 316T) bar as the electrode material. These bar electrodes were inserted into a machined piece of acetal plastic, with a 5mm-diameter hole drilled perpendicular to the electrode axis (Fig 12.2). The electrode surfaces were polished and the edges slightly burred to reduce irregularities that may cause high electric field stress.

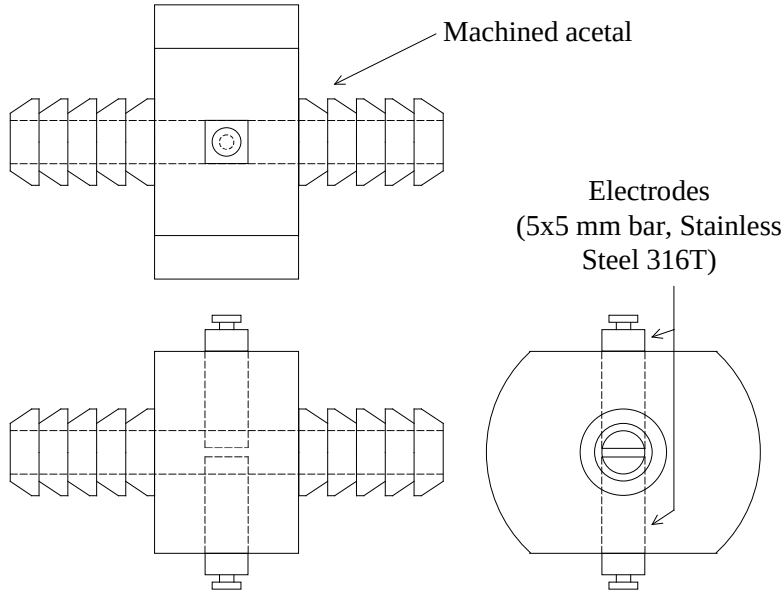


Figure 12.2 Electrode chamber

The electrodes were press-fitted into the acetal body. The gap between electrode surfaces is readily adjustable by using metal feeler gauges of various thicknesses. This gap, through which the water passes, determines the electric field strength for a given applied voltage. The gap used throughout these experiments was 0.65mm.

12.3 Microbiology

Both types of bacteria previously used in the testing of the 50Hz devices were used in this experiment. The bacteria *S. marcescens* was used for comparison with the 50Hz treatment results. The organisms were cultured in standard nutrient broth (NB) and incubated at 30°C.

The bacteria *E. coli* were also used in this experiment because of its relevance to water quality. It also allows more of a comparison with published PEF results as it features in many of the tests cited. *E. coli* was also cultured using standard nutrient broth (NB), and incubated at 37°C.

For both organisms, the inoculated broth was incubated overnight until reaching the early stationary growth phase (15 h). The bacteria were harvested by centrifugation and resuspended in sterile deionised water immediately prior to treatment. The conductivity of the resulting solution and thus power levels required for treatment were very similar throughout the experiments and thus have not been reported. The conductivity was approximately 2 μS/cm and the power 40 W.

Bacteria suspensions were added to 1.2 litres of deionised water and forced through the electrode chamber by a small submersible pump (Figure 12.3). An adjustable in-line valve enabled the flow rate to be varied. Treated and non-treated samples were taken immediately at the outlet of the electrode chamber. Each sample was collected with the electric field strength and flow rate adjusted to the correct value. The electric field was disabled before taking a control immediately after each sample.

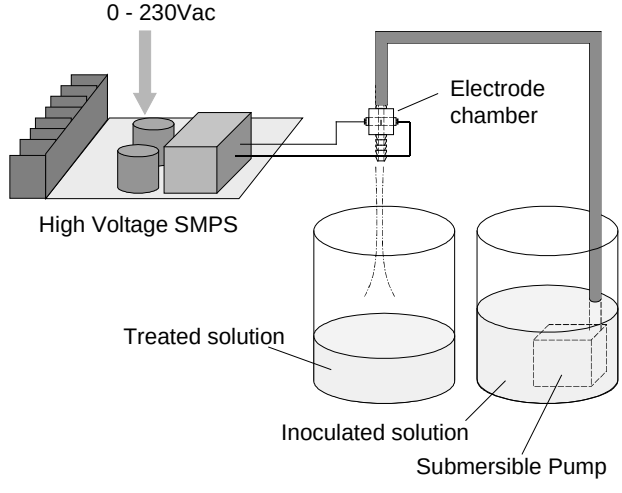


Figure 12.3 Experimental setup

Viability of the bacteria before and after treatment was assayed by counting colonies on an agar plate. The samples were first serially diluted with sterile deionised water and a known amount spread onto NA agar plates. The plates were incubated for 24 hours at the respective incubation temperature. The number of colony forming units (CFUs) was then counted. The dilutions were carried out in such a way as to give between 20 and 300 colonies per plate (except for very low concentration samples that had zero or very few colonies). Duplicate plates were used for each dilution.

12.4 Results

The survival ratio of *S. marcescens* was measured for the SMPS at an electric field

strength of 30 kV/cm, and varying exposure time (Figure 12.4). As exposure time is increased, the survival ratio decreases. However, the survival ratio decrease is not large despite a relatively large change in exposure time. The shortest exposure time is 0.34 ms, and this gives a survival ratio of 0.05 (95% lysing). It should be noted that the points shown are the results from a number of duplicated experiments undertaken over different days.

Figure 12.4 also shows the performance of the 50Hz device (Chapter 4). These results have been taken from a single experiment and are meant to be indicative only. At high exposure times the 50Hz waveform has a greater lysing effect than that of the SMPS. This may be because the peak field strength of the 50 Hz waveform is higher than that of the square wave SMPS and also the duration of each half cycle is much longer. At low exposure times the SMPS waveform is more effective. The low field regions in the 50Hz waveform reduce the effectiveness of this waveform at short exposure times.

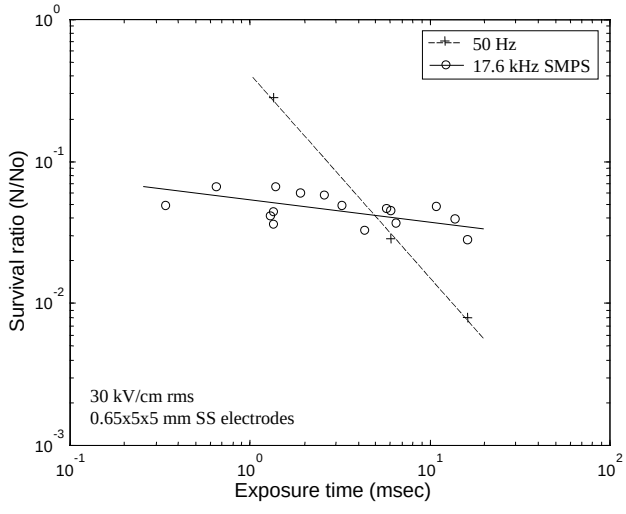


Figure 12.4 Survival ratio for *S. marcescens* at 30 kV/cm field strength

12.4.1 Results from E.coli Testing

SMPS single application experiments were repeated on *E. coli*. Figure 12.5 shows the results in comparison with *S. marcescens*.

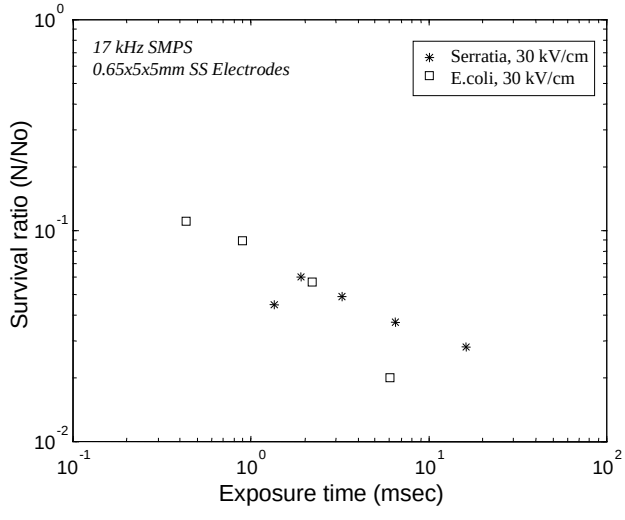


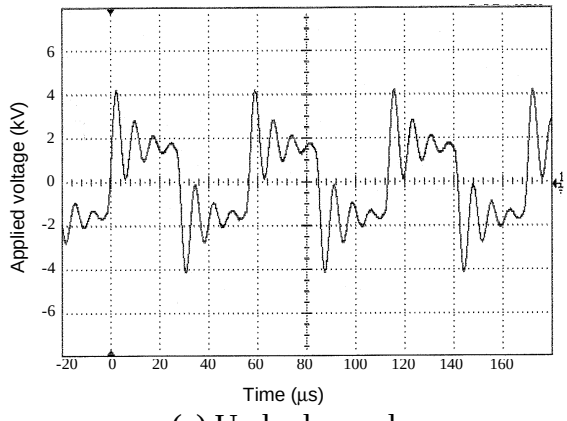
Figure 12.5 Survival ratio for *E. coli* and *S. marcescens* at 30 kV/cm

12.4.2 Effect of overshoot

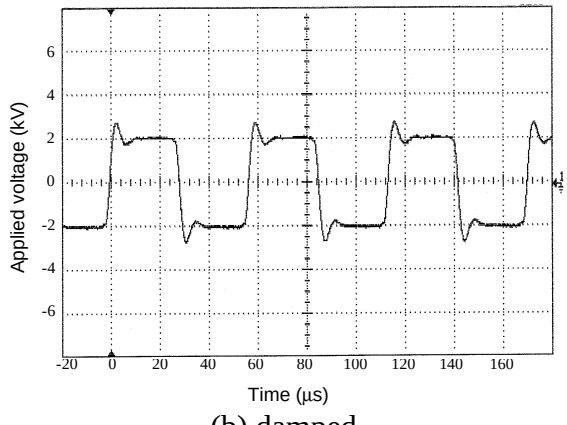
The load on the device has a high resistance since the water is deionised. This water load is a high output impedance for the SMPS which gives rise to a large amount of overshoot and ringing in the SMPS waveform (Figure 12.6(a)). The ringing is due to the resonance from the distributed capacitance and leakage inductance of the high voltage transformer. The transformer is underdamped. The damping factor of a pulse transformer may be described by Equation (12.1) [Nadkarni and Bhat 1985].

$$\delta = \frac{\sqrt{a}}{2}\left(\frac{R_1}{Z_0} + \frac{Z_0}{R_2}\right) \tag{12.1}$$

where $Z_0 = \sqrt{\frac{L_l}{C}}$ is the characteristic impedance of the transformer, R_1 is the total source impedance (primary winding resistance + external source impedance), R_2 is the sum of the load resistance and the secondary winding resistance referred back to the primary, and a is the attenuation factor, defined as $a = R_2/(R_1 + R_2)$. L_l is the leakage inductance of the transformer and C is the distributed capacitance referred to the primary. A transformer with δ<1 is highly damped, δ=1 is critically damped, and δ>1 is underdamped [Nadkarni and Bhat 1985].



(a) Underdamped



(b) damped

Figure 12.6 SMPS waveforms (a) underdamped (>100% overshoot) and (b) damped by high source impedance (20% overshoot)

It can be seen from Equation (12.1) that overshoot and ringing on the SMPS waveform may be damped by the addition of transformer source resistance. Figure 12.6(b) shows the damped waveform when a resistance of 11Ω is placed in series with the transformer primary.

To determine if the high voltage overshoot had any effect on the lysing of bacteria, experiments were performed using both waveforms. Figure 12.7 shows the results of this testing on *S. marcescens*. The waveform with the high overshoot had more effect in lysing bacteria than the one with a low overshoot. The difference, however, is not great. The waveform with little overshoot increased the survival ratio by an average of 0.2 log. It would seem that the level of damping does not make a significant difference to the survival ratio.

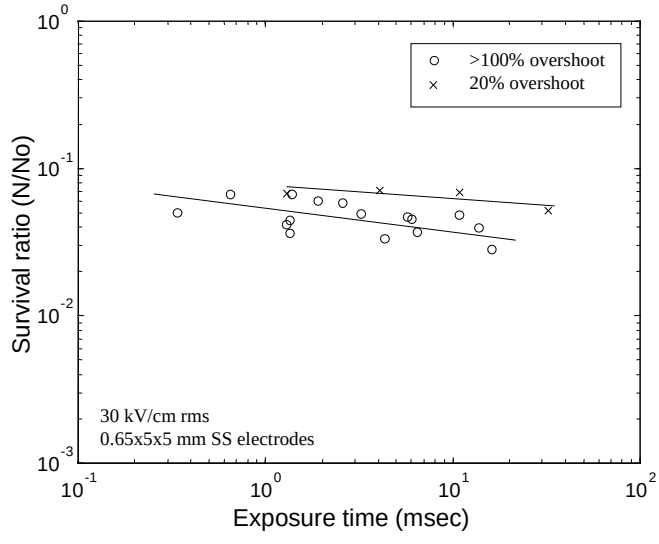


Figure 12.7 Effect of overshoot on the survival ratio of *S. marcescens*

A voltage waveform with high overshoot and ringing may cause problems in the operation of the device. The high voltage overshoots increase the stress on the transformer insulation and also contribute to electrode instability. The transformer operational lifetime may be reduced because of the insulation stress, and the electrodes may be more prone to arcing and damage.

In these experiments a primary series resistance was used to provide the damping. This method is inefficient and dissipates a large amount of power, which increases as the amount of current drawn increases. For this reason, a series resistance is not practical for a working model disinfection unit. Preferred methods of providing damping are to either optimise the transformer leakage inductance and distributed capacitance, or decrease the load impedance.

12.4.3 Effects of Variations in Electric Field Strength

The electric field strength was varied to ascertain its effect on survival ratio (Figure 12.8). It can be seen that as the field strength is increased, the survival ratio decreases. As expected, higher field strengths are more effective in reducing the numbers of bacteria. For an exposure time of 1.3 ms, the 20 kV/cm waveform survival ratio was 0.2 (80% lysing), as compared with the 40 kV/cm survival ratio of 0.04 (96% lysing).

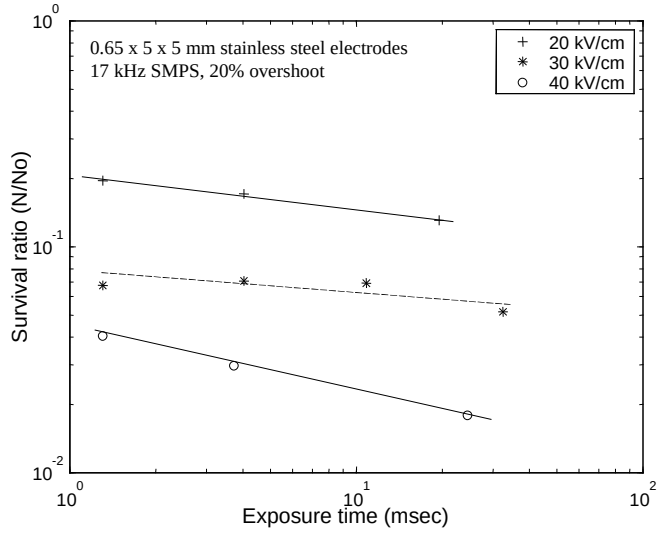


Figure 12.8 Effect of electric field strength on the survival ratio of *S. marcescens*

12.4.4 Multiple Applications

Since power and energy are directly proportional to the square of voltage, an applied field of 20 kV/cm would use 1/4 of the power of a 40 kV/cm field. From the results in Figure 12.8, the 20 kV/cm field at 1.3 ms exposure time gave a survival ratio of 0.2, whereas the 40 kV/cm field gave a survival ratio of 0.04 at the same exposure time. Hence if the 20 kV/cm waveform was applied twice, the survival ratio may match that of the 40 kV/cm waveform (0.2 x 0.2 = 0.04) and only use 1/2 the power. Therefore, it may be useful to use multiple applications of waveforms with lower magnitude to increase the efficiency of the device.

The overall efficiency of the treatment device may be increased by operating at a lower exposure time and/or voltage magnitude. The corresponding decrease in lysing rate may be compensated by the addition of a multiple number of electrodes.

Some multiple application tests were undertaken on both *E. coli* and *S. marcescens* to prove this idea. The results are shown in Figure 12.9.

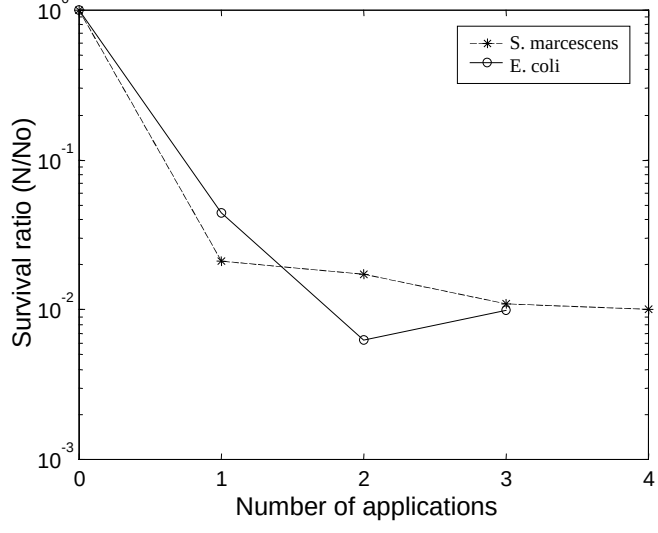


Figure 12.9 Multiple applications of SMPS treatment

The *E. coli* results show that successive treatments further reduce the survival ratio. However, there also seems to be a limit to the survival ratio, at which further applications have no added effect. The survival ratio limit for *E. coli* is about 0.01 (99% lysing rate). A similar limit is reached when multiple application tests are run on *S. marcescens*. The reason for the survival ratio limit is unclear, however a similar effect was seen in the initial 50Hz treatment results (Chapter 4).

12.5 Discussion

Although the SMPS is not as effective in reducing the numbers of bacteria as the previously cited 50Hz model, it does have significant advantages. The high voltage SMPS is much smaller, light weight and cheaper to manufacture than its 50Hz counterpart. The higher frequency used in the SMPS is electrically more stable than 50Hz (that is, the electrodes are much more robust to arcing). The SMPS does not need an air trap before the electrodes, whereas this is critical to the 50Hz device. Because the SMPS does not have 'dead' areas of low electric field, the exposure time and thus power may be reduced. Most of the experiments described in this paper used an exposure time of 1.3 ms, whereas the 50Hz treatment uses 17 ms. This 92% reduction in exposure time means an equivalent 92% reduction in energy used per volume of water. The reduction in energy needed to treat water may contribute to lower operating costs, but also may allow water of higher conductivity to be treated.

The lower performance of the SMPS in reducing the numbers of bacteria may be partially compensated by the use of multiple treatments (i.e. two electrodes in series). This will bring the performance level closer to the 50Hz performance level while still maintaining the lower energy levels and other benefits.

12.6 Conclusions

A high voltage water treatment device that operates at 50Hz AC has previously been presented. This device uses a 50Hz step-up transformer to provide the high voltage required. The large size and weight of this transformer has prompted design of a SMPS to replace it.

A high voltage SMPS was designed for use in water disinfection. The SMPS has a number of significant advantages over the previous 50Hz treatment device. It is able to reduce operating energy requirements by over 90%. It is also smaller, more lightweight and less expensive to manufacture.

The high voltage SMPS has been successfully tested on the bacteria *S. marcescens* and *E. coli*. The SMPS does not reduce the survival ratio of bacteria as much as the 50Hz treatment, but the exposure time and associated energy is significantly reduced. Using a number of SMPS treatment devices in series may further reduce the survival ratio.

Chapter 13

HIGH VOLTAGE WATER TREATMENT IN RECIRCULATING SYSTEMS

13.1 Introduction

Closed loop water recirculating systems that are open to outside contamination pose a risk to people in contact with those systems. Depending on many factors (temperature, susceptibility to outside contamination, flow rates in and out of the system, available nutrients, etc.), the growth of bacteria may lead to abnormally high levels which may be detrimental to health. The likelihood of bacterial growth in these systems also depends on the length of time the water has been sitting idle.

An example of a closed loop recirculating system is air conditioning. A given body of water is circulated through a building and acts as a heat exchange medium for controlling temperature within the building. In these systems, bacterial growth can occur and cause serious health concerns, especially when this involves certain pathogenic bacteria (for example, the genus *Legionella*). Many community outbreaks and even deaths have been associated with *Legionella* infections originating from air conditioning cooling towers [Mavridou *et al*. 1994, Keller *et al*. 1996, Bentham *et al*. 1993]. Most, if not all, systems rely on a residual disinfectant (eg. chlorine) to keep levels of these bacteria down.

It is feasible however, to install a continuous recirculating treatment system if this can effectively keep bacteria levels below minimum requirements. A given volume of water may be kept purified by extracting a stream at a given flow rate, treating it and returning it to the main body of water. The remixing means that the number of viable bacteria decreases exponentially as against an absolute reduction for single flow through of the entire reservoir. The total population of microorganisms is being reduced at a specific rate, determined by the effectiveness of the treatment, the total volume of water and the recirculation flow rate. This treatment may be effective in keeping the levels of microorganisms below acceptable levels, depending on the biological growth and multiplication rates within the system.

This chapter explores the use of high voltage in recirculating systems. In these applications, the outright effectiveness of the device is not as critical as in point-of-use applications. As bacteria in the water are circulated, they will experience a multiple number of applications, thus reducing their numbers to acceptable levels.

13.2 Theoretical Model

It is assumed that a recirculating system has a flow rate of Q m^3/s. The total volume of water is V m^3, most of which resides in a storage tank. The diverted flow (Q) is

electrically treated at an efficiency of *S* % (*S* % of bacteria flowing through the treatment chamber is rendered non-viable), then fed back into the storage tank. It is also assumed that uniform mixing occurs within the tank, and that the growth rate of bacteria within the tank is zero. This is acceptable if the recirculation flow rate is high compared to the total volume. The survival ratio of the bacteria may be given by the equation

$$\frac{N}{N_0} = e^{-\left(\frac{QS}{100V}t\right)} \tag{13.1}$$

where N is the total number of bacteria in the tank after recirculation time t (s), and N_0 is the original number of bacteria at time t=0. In a recirculation system, there are two things happening. Firstly, the % of bacteria lysed during a single pass depends on the flow rate or exposure time. This is already established in previous work [6]. Increasing the flow rate has less effect on the viability of bacteria passing through the chamber. Secondly, the increased flow rate (Q) means more water (and bacteria) passes through the treatment chamber in a given time. Thus, more bacteria are exposed to the electric field and total numbers are reduced more quickly than for slower flow rates.

Equation 13.1 has been presented in standard mks units. In this paper however, it is more convenient to use units which reflect the volumes and flow rates involved in actual tests. Hence throughout the rest of this paper, the flow rate, *Q*, is represented in ml/min (=1.7×10^{-8} m^3/s), the total volume, *V*, in ml (=1×10^{-6} m^3), and the time, *t*, in minutes.

If Equation 13.1 is plotted for a given flow rate and tank volume, the survival ratio decreases logarithmically at different rates, depending on the treatment efficiency (*S*). Figure 13.1 shows this plotted for *Q*=750 ml/min and *V*=1250 ml. This plot shows a much slower decrease in survival when the lysing rate is 50%. However, the decrease in survival is virtually the same when the lysing rate is 99% or above. This implies that in a closed loop system, such as an air conditioning system, a 99% lysing rate is just as effective in reducing the total numbers of bacteria as one with a 100% lysing rate and hence may be all that is required. A lysing rate of 90% is also quite effective in reducing the bacteria populations. This means that in the design of water treatment devices for a recirculating system, the flow rate may be increased at the expense of biological performance in order to make the overall system more effective.

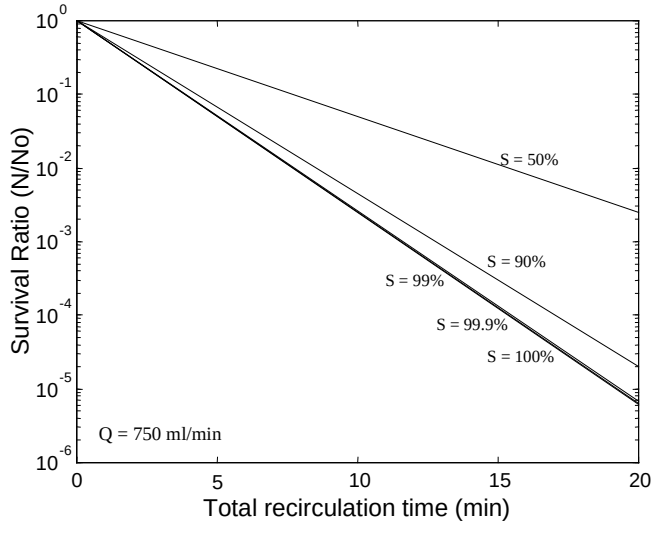


Figure 13.1 Theoretical reduction in bacterial population for various lysing rates

Figure 13.2 shows Equation 13.1 plotted for various lysing rates and flow rates. It can be seen from this graph that increasing the flow rate is a more effective way of reducing the total bacteria population than increasing the specific lysing rate. The survival ratio curve for a flow rate of 750 ml/min and lysing rate of 50% is identical to that for half the flow rate (375 ml/min) and a 100% lysing rate. In most physical disinfection systems (eg. electric field treatment, ultra-violet radiation) the lysing rate is dependent on the exposure time. What this graph shows is that in order to maximise the effectiveness of disinfection in a recirculating system, the exposure time should be decreased at the expense of lysing rate (ie. use a higher flow rate than that specified for maximum lysing). There should be some optimum operating conditions, which will provide the most effective system.

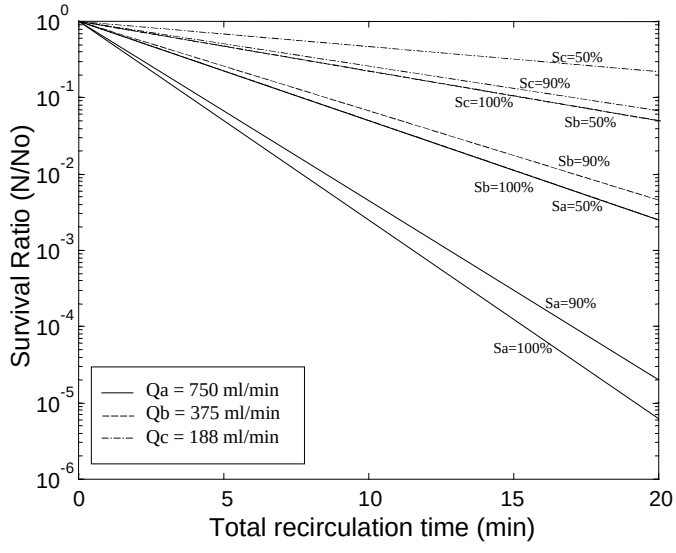


Figure 13.2 Theoretical reduction in bacterial population in a recirculating system for various lysing rates and flow rates

For high voltage treatment, the energy density required to reduce the levels of bacteria by 3 log (0.001 survival, or 99.9% lysing) may be calculated for different exposure times (or flow rates). The use of energy density is to show that increasing the recirculation flow rate not only reduces the total numbers of bacteria more quickly, but may also add to energy savings. The amount of power used by the water load is dependent on the water conductivity. The resistance of the water load may be calculated from the equation

$$R = \frac{g}{\kappa A} \tag{13.2}$$

where g is the electrode gap (cm), κ is the water conductivity (S/cm), and A is the electrode area (cm^2). At a frequency of 17 kHz, the load capacitance is 27 pF. The capacitive reactance is 344 kΩ. The real power and hence energy loss consumed by the water load is due to the resistance only. The power consumed by the capacitance of the water is purely reactive. Hence, the average energy dissipation of the capacitance is zero. Since the capacitive current is at 90 degrees to the resistive current, the resulting total current has a slightly higher magnitude than if only a resistive load is considered.

At a water conductivity of 2 μS/cm the resistive current is 15.4 mA and the total current 16.5 mA. Thus, the capacitance adds to the magnitude of the current flowing through the circuit and will slightly increase I^2R losses in the SMPS. The resistive power consumed by the water load is

$$P = E^2 g \kappa A \tag{13.3}$$

where E is the applied electric field (V/cm). The energy density may be calculated from the equation

$$W = Pt_c / V \tag{13.4}$$

and t_c is the recirculation time required to reach the required level of disinfection, and may be calculated from Equation 13.1.

Figure 13.3 shows the survival ratio of *E. coli* when exposed to a high voltage from the SMPS (Chapter 12). The survival ratio increases as the exposure time is reduced. The exposure time is defined as the length of time a particle of water is exposed to the electric field during a single pass. For a given electrode dimension the exposure time is directly related to the flow rate. The energy density required to reduce the *E. coli* population by 3-log is calculated for each different exposure time and also plotted. The energy density required reduces with decreasing exposure time, that is, it is more efficient to run the device at high flow rates and low exposure times. Although *E. coli* passing through the electrodes are lysed at a lower rate, the increased flow rate means that the frequency of exposure is increased and hence the overall numbers of *E. coli* are reduced more quickly.

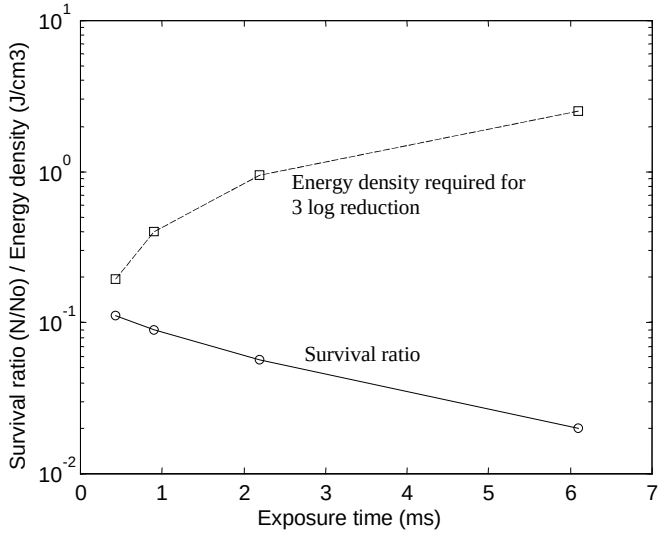


Figure 13.3 Survival ratio for single SMPS application versus the calculated energy density required to reduce total *E. coli* levels by 3 log. Water conductivity in these tests was 2 μS/cm, and the average electric field strength was 30 kV/cm.

The energy density values in Figure 13.3 have been included as a comparison to show the increased efficiency of reducing the exposure time by increasing the recirculation flow rate. The values shown have been calculated for a water conductivity of 2 μS/cm. For high voltage treatment the energy density is proportional to the water

conductivity. Hence for commercial recirculation systems to use this technology it may be necessary to control the water conductivity by use of a deionisation column or other similar method.

13.3 Experimental Methods

Recirculation tests were performed on bacteria *E. coli* using the apparatus shown in Figure 13.4. A small sample of viable *E. coli* cells were suspended in nutrient broth (NB) and incubated overnight at 37°C. The resulting cells were harvested by centrifugation and resuspended in 1250 ml sterile deionised water. The conductivity of the resulting suspension was approximately 2 µS/cm. A submersible pump was used to recirculate the inoculated water via a set of electrodes energised from a high voltage supply. The electrodes are made of stainless steel 316T, and have surface dimensions 5x5mm. They are separated by a 0.65mm gap. The peak flow rate used in these experiments was 750 ml/min. The exposure time at this flow rate is 1.3 ms, and the water velocity through the electrodes is 3.8 m/s. The electrodes are identical to those used in previous testing (Chapter 12). The inoculated solution was stirred throughout the experiment using a magnetic stirrer, as also shown in Figure 13.4.

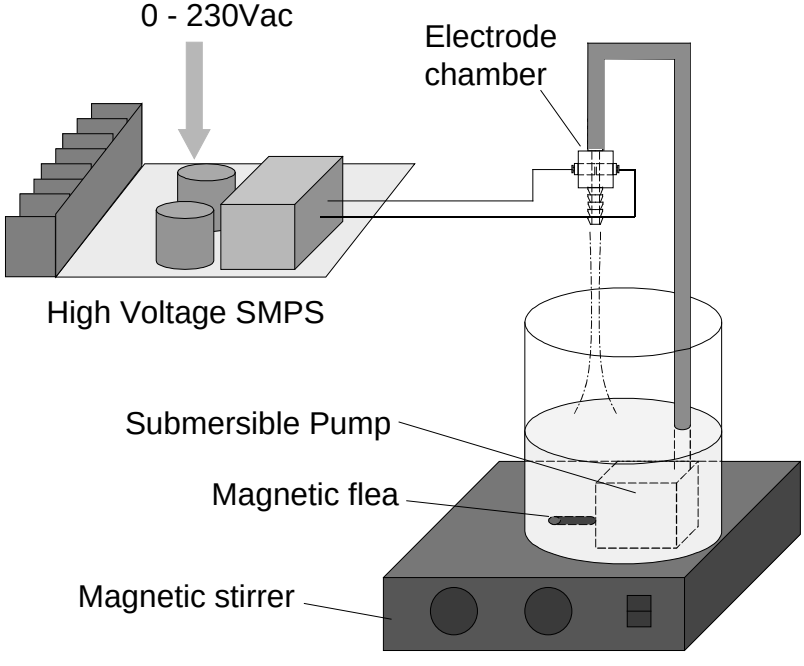


Figure 13.4 Recirculation test equipment

Water samples were taken at start-up and at consecutive 5-minute intervals. Each sample consisted of a treated sample, and an untreated sample. Immediately after each treated sample, the high voltage supply was disabled, an untreated sample taken, and the high voltage switched on again. This method gave information on the concentration of bacteria with time (untreated sample), and the lysing rate at each 5-minute interval (treated vs untreated samples).

Samples were serially diluted with sterile deionised water and plated on nutrient agar. The plates were incubated for 24 hours and the number of colony forming units

(CFUs) counted to provide the bacteria concentration of the sampled solution. Duplicate plates were made of each sample dilution, and the average of these is reported.

13.4 Results

The experimental results of the recirculation tests on *E. coli* are shown in Figure 13.5. The electrodes were energised from a 17 kHz SMPS. At a flow rate of 375 ml/min and an electric field of 25 kV/cm, the average lysing rate measured throughout the experiment was 99%. The corresponding survival ratio for this test closely matches that of the theoretical values.

At a flow rate of 750 ml/min and electric field strength of 25 kV/cm the measured average lysing rate was also about 99%. For the same flow rate and increased electric field strength of 30 kV/cm, the measured average lysing rate was 99.8%. The survival ratio curve for each of these tests closely follows each other. This agrees with the theoretical result that lysing rates over 99% have similar curves. Both tests deviate only slightly from the theoretical model, as shown in Figure 13.5.

Reducing the exposure time by a factor of 2 (doubling the flow rate) reduces the survival ratio by three orders of magnitude. Increasing the flow rate has a greater effect on decreasing total bacteria numbers. This is a counter-intuitive result and is an important characteristic of this treatment system.

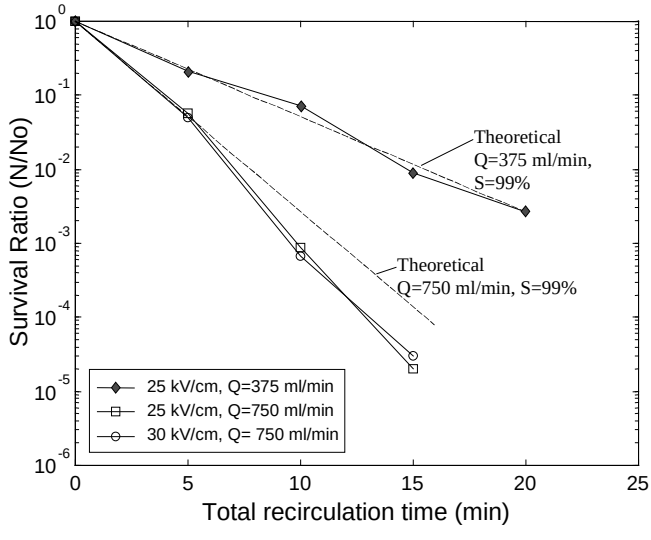


Figure 13.5 Recirculation test results on *E. coli*, compared with theoretical values.

13.5 Legionella Testing

As most health concerns in air conditioning systems involve the bacteria *Legionella*, an experiment was performed to measure the effects of high voltage exposure on the viability of this organism.

The experimental equipment was setup as shown in Figure 13.6. Water from the municipal supply first passed through a mixed bed deionising column. This reduced the conductivity of the water to <1μS/cm. The deionised water then passed through a 10 litre-per-minute 50 Hz treatment chamber with the high voltage applied. The reason for using 50 Hz rather than the SMPS was that the 50 Hz system was readily available and

would treat the higher flow rates typically used in air conditioning systems. The electrodes used were concentric cylinders with a separation gap of 0.18 cm. A 50 Hz voltage of magnitude 5.5 kV rms was applied to the electrodes to give an electric field strength of 30 kV/cm rms.

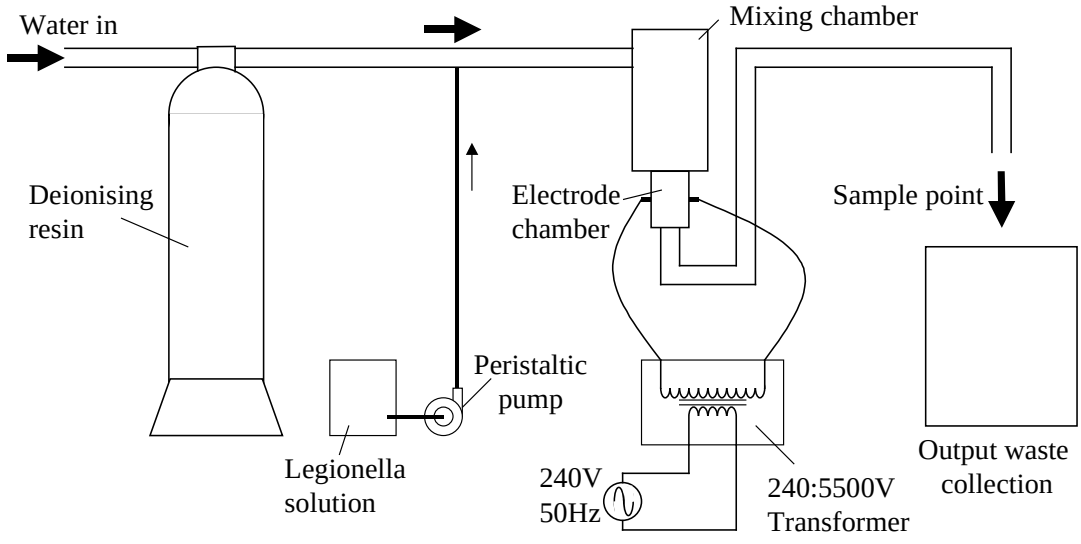


Figure 13.6 *Legionella* test equipment

The test organism was *Legionella rubrilucens* (NZRM 2955). The bacteria were washed and resuspended twice in deionised water. The inoculated water was injected into the system between the deionising resin and the electrode chamber. A peristaltic pump was used to inject the bacteria at a constant rate. After 30 seconds of injection the levels of bacteria in the system are assumed to be uniform. An output sample was taken at this stage. Immediately following this sample, the high voltage was disabled and five seconds later a control (untreated) sample taken. A 'blank' sample and control were also taken with no addition of *Legionella*. This was to ascertain the background contamination of the water supply. An independent laboratory processed the samples. Results are shown in Table 13.1.

A portion of each sample was subjected to heat treatment due to the possible presence of background organisms. Untreated samples were plated in duplicate and heat treated samples were plated in triplicate. The results reported are the averages of the duplicate or triplicate counts. The samples with no heat treatment gave a 99.7% reduction in viability after the high voltage application. The samples subjected to heat treatment to remove background bacteria gave a 99.8% reduction in *Legionella*.

	Untreated Sample		**Heat Treated Sample**	
Sample	**Non-LLC cfu/mL**	**LLC cfu/mL**	**Non-LLC cfu/mL**	**LLC cfu/mL**
Blank no HV	$1.00x10^2$	<10	$1.60x10^1$	<1
Blank with HV	$1.50x10^1$	<10	$6.00x10^0$	<1
Test no HV	$1.20x10^3$	$7.60x10^5$	$5.67x10^2$	$6.70x10^5$
Test with HV	$1.08x10^3$	$2.50x10^3$	$8.50x10^1$	$1.44x10^3$

Table 13.1 *Legionella* test results. LLC = *Legionella* like colonies.

These tests on *Legionella* suggest the application of high voltage water treatment to air conditioning systems may be successful. The lysing rate of a 50 Hz treatment system has been shown to be adequate (99.7%) for recirculation systems such as this. It also suggests that further development of the SMPS may be applicable for commercial air conditioning systems.

13.6 Conclusions

This paper has looked into the application of a point-of-use water treatment device in recirculating systems. The water treatment device uses high voltage to destroy microorganisms suspended in deionised water. A theoretical model is presented which describes the logarithmic reduction in levels of bacteria in the water over time. This model assumes that the treatment effectiveness of the device remains constant over the duration of the experiment. This assumption is validated by experimental results. The lysing rate of the device on *E. coli* remains constant throughout the duration of the experiment.

It can be seen from the theoretical model that the effectiveness of a point-of-use treatment device does not have to be large in order to give good results. A lysing rate of 99% achieves a similar overall performance to a device with a 100% lysing rate. Indeed, the most effective means of increasing the overall performance is not to increase the performance of the treatment device, but to increase the recirculation flow rate. This means that for these types of systems, the flow rate through a point-of-use treatment device may be increased, even with a corresponding decrease in effectiveness, and still perform more effectively. Consequently, the minimum energy requirements for a high voltage SMPS to destroy 99.9% of total bacteria in solution occurs at the lowest exposure time. It may even be possible to further reduce the exposure time and increase the efficiency of this treatment method.

Recirculation experiments have been performed on the bacteria *E. coli* using high voltage from a SMPS. The experimental results closely agreed with values calculated from the theoretical model.

The effectiveness of a 50 Hz high voltage treatment on the viability of *Legionella* has also been tested. The high voltage exposure reduced the viability of *Legionella* by 99.7%. This indicates that a high voltage water treatment system should be effective in closed loop systems such as air conditioning.

Chapter 14

EFFECTS OF CELL GROWTH PHASE ON THE INACTIVATION OF BACTERIA USING HIGH VOLTAGE

14.1 Introduction

A few researchers have looked into the effects of cell growth stage on the effectiveness of the PEF treatment [Hulsheger *et al*. 1983, Pothakamury *et al*. 1996, Schoenbach *et al*. 1997, Wouters *et al*. 1999]. All of them have found that to varying degrees, cells harvested in the growth phase are more susceptible to the electric fields than cells harvested in the late stationary phase. Pothakamury *et al.* (1996) looked into the effects of growth stage in some detail. They treated the bacteria *Escherichia coli* harvested at various stages of growth with high voltage PEF. They found that exponential-stage cells were more sensitive than stationary-stage and lag-stage cells to the PEF treatment. None of these authors offer any explanation or discussion for this observed difference. Most of the authors treat the finding as just another factor influencing the rate of cell inactivation.

This chapter examines the effects of cell growth stage on the performance of high voltage water treatment systems. It discusses the implications of these findings on the commercial development of such a technology. It also raises questions over standard laboratory procedures that are widely used in the testing and certification of water treatment devices.

14.2 Materials and Methods

Two types of bacteria were used in these experiments. *S. marcescens* were used to provide a comparison to earlier results, and *E. coli* were used to give a comparison with some of the cited PEF treatment results. Both organisms were grown in standard nutrient broth. *S. marcescens* were incubated at 30°C, and *E. coli* at 37°C.

A small amount of a bacterial colony from a nutrient agar plate was suspended in nutrient broth and incubated at the appropriate temperature in a shaking water bath. After 24hrs, 0.1ml of solution was taken and added to 9.9ml of fresh nutrient broth. This broth was incubated at the appropriate temperature and samples taken at regular intervals over a period of days to measure the growth rate for each bacteria. Each sample was serially diluted and plated onto nutrient agar. Samples were plated in duplicate and the average number of colonies reported. In this way the growth curve was plotted. Known concentrations of samples, taken at various points along the curve, were treated using the high voltage SMPS. The electric field strength used in these experiments was 30 kV/cm and the exposure time was 1.6 msec.

At each treatment point an amount of inoculated broth (1ml for early growth stage, and 0.2ml for later stages to give roughly equivalent concentrations) was added to one litre of sterile deionised water. The water was then pumped through the electrode chamber and collected in a sterile container (Figure 14.1). Samples were taken before and after treatment. The pump, electrode and pipes were then disinfected using chlorine. The chlorine was drained and the system extensively flushed three times to remove all traces of chlorine. The system was then flushed with sterile deionised water to remove ionic residue. The treated water was again pumped through the electrodes for a second treatment. The chlorination and rinsing process was repeated and a third treatment undertaken. The time between high voltage treatments was approximately 15 minutes.

Three consecutive applications of the high voltage were taken in order to test if the second and third applications reduce cell numbers with an equal efficiency as the initial treatment. This test would also indicate whether there was a proportion of the bacteria that are inherently resistant to this treatment method.

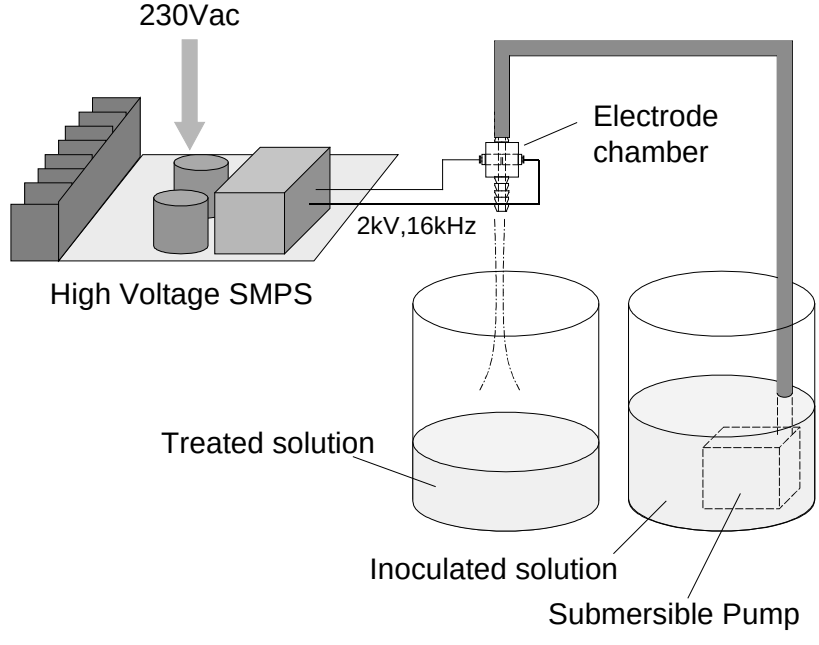


Figure 14.1 Experimental test equipment

14.3 Results

14.3.1 Testing on *S. marcescens*

The initial broth concentration of *S. marcescens* for each experiment at different incubation times is shown in Figure 14.2. This shows the exponential growth phase, stationary phase and decline phase. The boundaries between the phases are at approximately 20hrs and 200hrs. The initial lag phase (where the numbers of bacteria remain constant before the exponential growth begins) lasts for only the first hour.

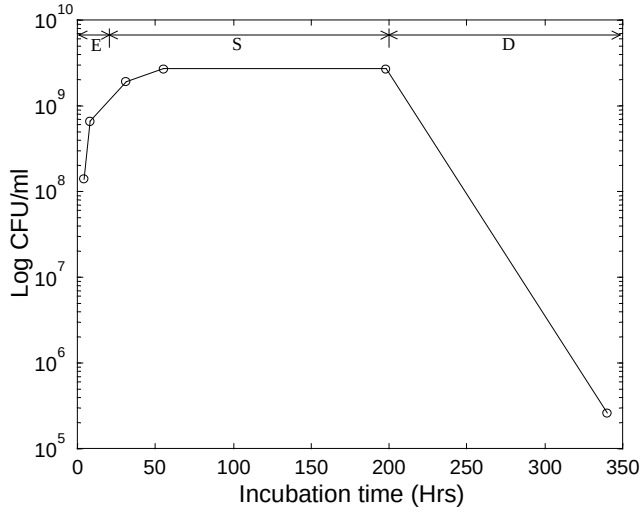
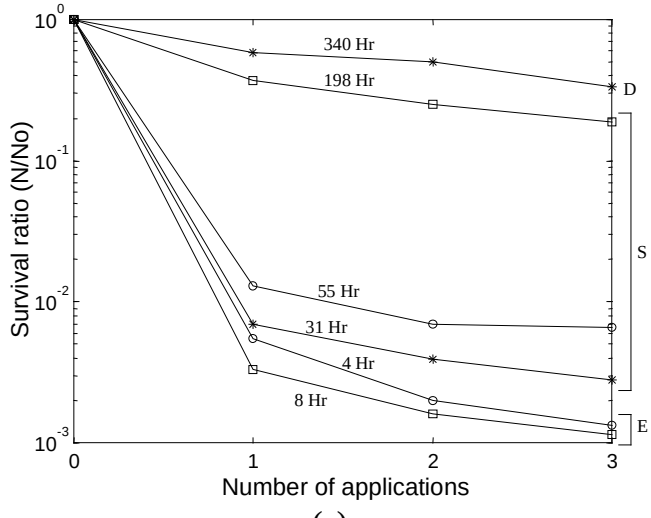
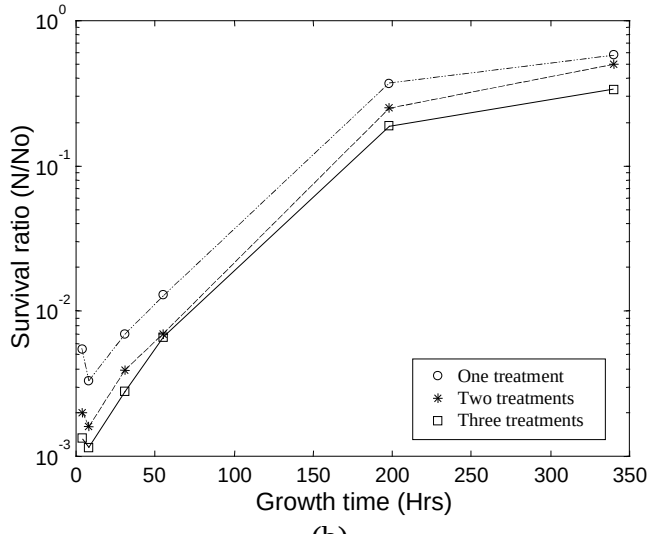


Figure 14.2 Initial broth concentration of *S. marcescens* at different incubation times, showing (E) exponential growth phase, (S) stationary phase, and (D) decline phase.



(a)



(b)

Figure 14.3 (a) Survival ratio of *S. marcescens* for multiple applications and various incubation times. (E) is the exponential growth phase, (S) stationary growth phase and (D) decline phase. **(b)** Survival ratio for each experiment plotted against growth time.

The results for multiple high voltage treatment tests for various exposure times are shown in Figure 14.3(a). As the incubation time is increased, the survival ratio also increases. Bacteria harvested during the logarithmic growth stage (4 & 8 hours) had a higher degree of death (~0.001 survival, or 99.9% reduction). In the late stationary and decline phases the survival ratio is greater than 0.1 (<90% reduction). There is also a large difference in survival between cells harvested in the early stationary phase and those harvested in the late stationary phase. Experimental error bars were not included in this graph because in most cases the statistical error was insignificant.

The second and third applications have less effect than the first application. For all experiments there seems to be a saturation limit, where the survival ratio will not decrease any further. This suggests that for each experiment a certain proportion of the bacteria seem resistant to treatment, even when the treatment is applied a number of times. This is shown more clearly in Figure 14.3(b). It shows that the first application of high voltage reduces the numbers of bacteria by varying amounts, depending on length of incubation time. However, the second and third applications further reduce the numbers of bacteria by roughly the same amount independent of growth time.

14.3.2 Testing on *E. coli*

The measured growth curve for *E. coli* is shown in Figure 14.4. The lag stage lasts for the first few hours [unpublished data] and is followed by the logarithmic growth and stationary phases. The decline phase was not reached in this experiment due to contamination of the incubated culture.

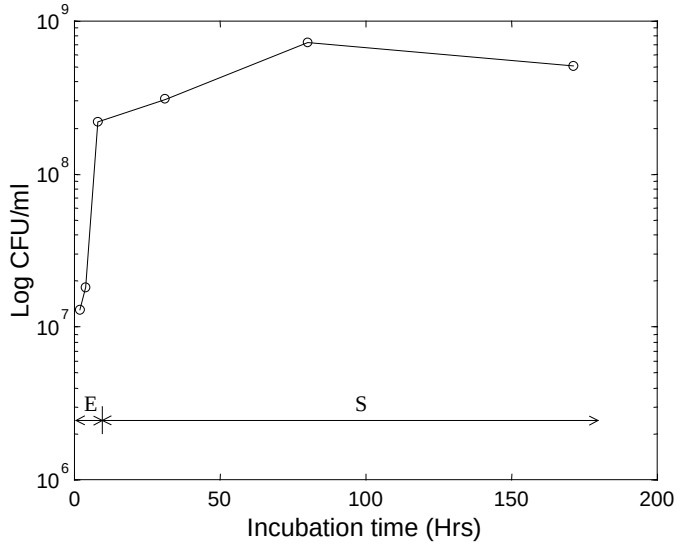
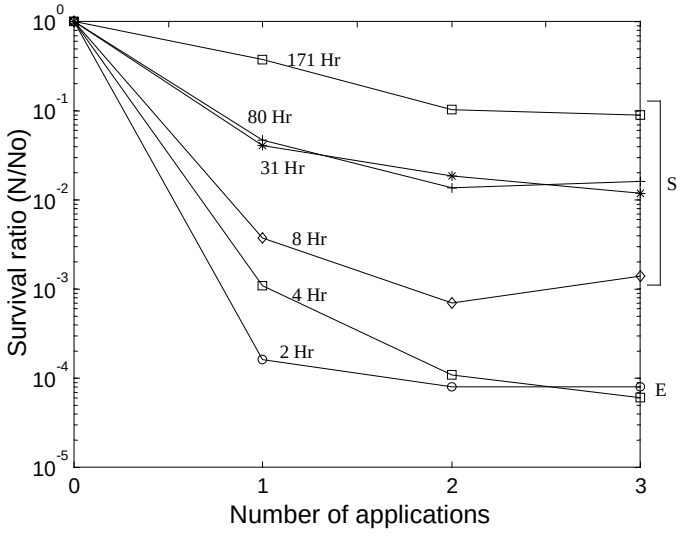
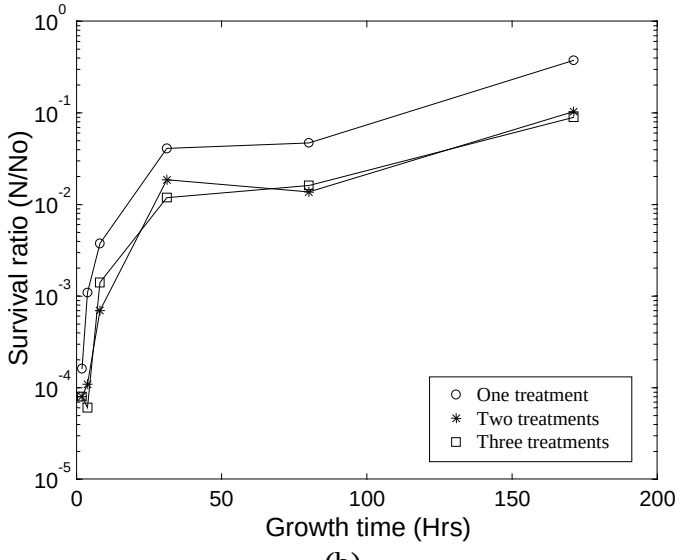


Figure 14.4 Initial broth concentration of *E. coli* at different incubation times, showing (E) exponential growth phase, and (S) stationary phase.

Figure 14.5(a) shows the reduction in survival ratio for one, two and three applications of the high voltage SMPS. The curves are very similar to that for *S. marcescens* (Figure 14.3(a)). As the incubation period is increased, so too does the survival ratio. Bacteria harvested at 2 and 4 hours were non-culturable after two and three applications respectively (the levels of surviving bacteria were below detectable limits). After 171 hours of incubation, however, 10 percent of the bacteria survived three applications.



(a)



(b)

Figure 14.5 (a) Survival ratio of *E. coli* for multiple applications and various incubation times. (E) is the early exponential growth phase, and (S) stationary growth phase **(b)** Survival ratio for each experiment plotted against growth time.

The second and third applications do not have as much effect in reducing the survival ratio as the first application, as shown in Figure 14.5(b). The reduction in survival ratio reaches a limit, which is different for each growth stage. It would seem that a proportion of the *E. coli* population is inherently resistant to the high voltage SMPS treatment, the amount depending on the growth stage. It also shows that there is perhaps some justification in a second treatment, but little effect of a third.

14.4 Testing on Natural Microfauna

Bacteria in natural water supplies will predominantly be in a dormant or non-metabolising state and therefore show higher resistance to applied electric fields. In order to test this theory, four water samples were taken from natural sources with a reasonably high amount of contamination. Two samples were taken from duck ponds at

the local botanical gardens; another sample was taken from a river immediately adjacent to the gardens, and a fourth taken from a small urban stream that flows through the University of Canterbury. A resident population of ducks and other natural wildlife are present at each of these water sources. Samples were also taken immediately following a heavy fall of rain. Both of these factors implied the samples were contaminated with a high number of naturally occurring bacteria.

The water samples were diluted with sterile deionised water to give a uniform conductivity of 25 ± 5 μS/cm. Lowering of the conductivity to this level is necessary to enable the water to be treated directly by the high voltage SMPS. The samples were then exposed to the high voltage SMPS. The electric field strength was 27 ± 3 kV/cm, and the exposure time 1.35 msec. Samples pre- and post-treatment were plated onto nutrient agar and incubated at either 20°C or 37°C for up to 72 hours. The general number of colonies that appeared after incubation was counted. These colonies consist of a wide range of natural microfauna that grow at these temperatures. Duplicate plates were made of each sample and the average reported. A summary of results is shown in Table 14.1.

Water source	20°C Incubation		37°C Incubation	
	Pre-treatment (CFU / 100mL)	Reduction (%)	Pre-treatment (CFU / 100mL)	Reduction (%)
A. Gardens pond #1	253,000	16	54,500	39
B. Gardens pond #2	154,000	29	13,000	35
C. Avon river	253,000	47	35,000	10
D. University stream	104,000	85	11,000	76

Table 14.1 Testing the high voltage SMPS on natural microfauna.

There is a reasonably high level of contamination in all four water supplies. There are high numbers of organisms that grow at 20°C and substantially less that grow at 37°C. The initial numbers of organisms in the supplies are even higher considering the pre-treatment levels listed in Table 1 have been taken after dilution with deionised water. The amount of dilution depended on the conductivity of the source water, and was one part source to between 3 and 7 parts deionised water.

The reduction in viable colonies after one treatment with the high voltage ranges from between 10 and 85%. This is a relatively low amount of reduction (<1-log) compared to the results for laboratory-grown *S. marcescens* and *E. coli*, shown in Figures 14.3 and 14.5. These organisms, harvested in exponential or early stationary phase, can be reduced by >2-log with application of high voltage with similar parameters. When compared to Figures 14.3 and 14.5, the results of the tests on general microfauna indicate most organisms may be in the late stationary or decline phase of growth, as predicted by the initial theory. This may be the reason for the low reduction in viability.

14.5 Discussion

The effect of high voltage on the viability of both *S. marcescens* and *E. coli* depends strongly on incubation period. Bacteria harvested in the exponential growth phase were less resistant to the electric field than when harvested in the later stationary

phase. For both bacteria harvested in the exponential growth phase, nearly all viable bacteria could be destroyed (>99.99% reduction for *E. coli*, Figure 14.5(a)). Cells harvested in the decline phase were the most resistant. For *S. marcescens* harvested in the decline phase the maximum reduction achieved after three applications of high voltage was <70%.

In the early exponential growth phase, almost all cells are synchronised in a process of active growth. The exponential-stationary phase transition involves a period of unbalanced growth during which the various cellular components are synthesised at unequal rates. During the stationary phase, the growth rate is exactly equal to the death rate. During the decline phase the death rate becomes higher than the growth rate, and the number of viable cells decline. The longer the incubation period the smaller the proportion of bacteria that are in a state of growth. Thus the treatment results would strongly suggest that bacteria in a state of growth are susceptible to high voltage treatment, whereas those not growing are inherently resistant.

Most tests using PEF treatment have been undertaken on bacteria in their early stationary phase where there is unbalanced growth [Castro *et al*. 1993, Grahl and Markl 1996, Hulsheger *et al*. 1993, Jayaram *et al.* 1992b & 1993, Jayamkondan *et al*. 1999, Matsumoto *et al*. 1991, Mazurek *et al*. 1995, Mizuno and Hori, 1988, Palaniappan and Sastry 1990, Pothakamury *et al*. 1996, Qin *et al*. 1994 & 1995, Schoenbach *et al*. 1997, Wouters *et al*. 1999]. This may account for some of the wide variations in results. Also, many of the PEF tests have used liquid foods as the suspension medium. These liquids provide a good source of nutrients and are conducive to bacterial growth. The inlet temperature for these experiments is usually between 20 and 40°C, also conducive to bacterial growth. As soon as the bacteria to be tested are introduced to the suspension medium, it must be assumed that the lag phase will begin. Depending on the length of time before the high voltage PEF testing begins the cells may have commenced growth. This would cause the bacteria to be susceptible to the electric field, and thus the high levels of reduction reported (up to 9-log).

In the *E. coli* experiments presented in this paper, cells harvested in the late lag phase/ early exponential phase (2 hours growth) were also highly susceptible to the electric field treatment. At the start of the incubation period, cells in the stationary phase were added to the nutrient broth. At some point during the lag phase these cells went from being a mixture of resistant and non-resistant to becoming almost all non-resistant. Where exactly this transition takes place is as yet uncertain.

The treatment mechanism is a physical process in which the electric field attacks the cell membrane. The role of the cell wall is to provide rigidity and strength to the cell membrane. This has been shown to effect or limit pore formation in the membrane when undergoing electroporation. Chang (1992) studied the formation of micropores in the membrane of cells due to electroporation. He froze the cells at various time periods following application of the high voltage, and studied the membranes under an electron microscope. He found that the openings in the cell wall itself limited the maximum expansion size of the micropores. The membrane holes expanded to a size equivalent to the average size of openings in the cell wall. It may be that in the growth process, changes are made to the cell wall as nutrients become depleted and cells slow their metabolism. These cell wall changes have the effect of making the cell more resistant to the electric field by further protecting the membrane.

High voltage (50 Hz) water treatment has previously been tested on the cysts of *Giardia intestinalis* (Chapter 6). These cysts are relatively hardy and are protected by a thick cell wall structure. However, all cysts that were observed after treatment with the high voltage were destroyed. Thus *Giardia* cysts in their dormant state do not have

resistance to the high voltage. This suggests that there must be some fundamental difference in the physical structure between these cysts and bacteria that is attributing to the different responses.

The tests on bacteria for both the PEF treatment and the 50Hz and SMPS treatments follow standard microbiological procedures. It is common to grow the bacteria to be tested on or in an appropriate media at a specific temperature. The bacteria are harvested when they reach the early stationary growth phase (the bacteria numbers are then high enough for accurate results), then prepared (injected or suspended into the appropriate solution) and put through the device to be tested. Numbers of viable bacteria are then assayed on the appropriate growth media before and after treatment to measure the effects of the treatment.

These test methods are incorporated into national standards for testing the performance of commercial water purifiers, such as that for Australia and New Zealand [AS/NZS 4348, 1995]. These standard test methods are perhaps not appropriate for high voltage treatment devices. Since the performance of such devices is strongly influenced by growth phase, standard procedures will give misleading results. Bacteria in natural water supplies would normally be in a non-growing or dormant state, and thus will be reasonably resistant to high voltage disinfection. The physiological state of these naturally occurring bacteria will be different to those cultured in a laboratory. A high voltage disinfection system may be tested by a laboratory and found to be effective, whereas in the actual working environment it may perform less effectively.

The effects of growth phase on the survival of bacteria undergoing treatment with high voltage as been studied. For both *S. marcescens* and *E. coli*, the numbers of surviving bacteria after treatment strongly depended upon the length of time they were incubated. As the incubation time was increased, the survival ratio also increased. Bacteria harvested in the logarithmic growth and early stationary phases were much more susceptible to the high voltage treatment than bacteria harvested at the late stationary and decline phases. This strongly indicates that bacteria harvested while in an active state of growth are susceptible to this treatment, whereas mature bacteria are resistant. Rather than the growth-state of the bacteria being one of many factors contributing to the effectiveness of high voltage treatment systems, it is instead critical to the very success of these systems.

Non-metabolising cells seem to have a protective mechanism that shields them from the effects of the electric field. Further work in this field is needed to isolate the physical or chemical mechanisms by which this occurs. Better understanding of this protective mechanism is important so that new methods may be designed to overcome it.

Chapter 15

DISCUSSION AND FURTHER WORK

High voltage treatment technology has been developed in this thesis. However, the low performance of the devices and some variations in some results could be explained by the effect of cell growth stage, as documented in Chapter 14. The physiological state of the bacteria being treated plays an overwhelming role in the effects of high voltage treatment. Bacteria that are actively metabolising seem to be susceptible to high voltage treatment, whereas those in a non-metabolising state are resistant. In actual water supplies bacteria are mostly in a non-metabolising state and as such are inherently resistant to electric fields of these magnitudes. Thus the high voltage treatment method may be ineffective on these supplies due to the low amount of bacteria reduction, unless significant improvements are made.

High voltage treatment of liquid foods may be effective because of the high nutrient loading and ideal conditions for bacteria proliferation. It is because of this very fact that foods require treatment in the first place. Due to the high conductivity of these liquids however, constant high voltage treatment systems are unlikely because of the high current densities and high power required. These types of liquids lend themselves more to PEF treatment systems where the applied energy is limited and delivered in bursts.

15.1 Summary of cited PEF treatment on *E. coli*.

Some of the work described in this thesis has been undertaken on the bacteria *E. coli*, for comparison with cited PEF literature. A summary of the published work on the effect of PEF on these bacteria is listed in Table 15.1. These included references from nine different authors [Dunn and Pearlman 1987, Evrendilek *et al*. 1999, Grahl *et al*. 1992, Hamilton and Sale 1967, Hulsheger *et al*. 1983, Martin *et al*. 1997, Martin-Belloso *et al*. 1997, Matsumoto *et al*. 1991, and Qin *et al*. 1994]. These authors applied PEF to *E. coli* suspended in a variety of media, including milk, fruit juice, saline solution, phosphate buffer and even liquid egg. No cited references use natural or pure water as a suspension medium.

These results may be compared to the high voltage treatment described in this thesis. The high voltage SMPS obtained a 1-log reduction of *E. coli* for a single application at the lowest duration time of 400 μs and 30 kV/cm peak field strength (Figure 12.5).

The deionised water used in these experiments has a much lower conductivity than the liquid foods used in the PEF treatments. This means the current density is also much lower and this may contribute to the reduced effectiveness, as is further explained in Section 15.3.

Researchers	Media	Log reduction	Peak electric field (kV/cm)	No. of pulses	Pulse duration (μs)	Total exposure (μs)
Dunn and Pearlman (1987)	Milk	3	28	23	100	2300
Evrendilek *et al*. (1999)	Apple juice	5	30	43	4	172
Grahl *et al*. (1992)	Milk	3	22	5	40	200
Hamilton and Sale (1967)	Saline (0.1%)	2	17	10	20	200
Hulsheger *et al*. (1983) 4h incubation	Phosphate buffer	4	18	30	36	1080
Hulsheger *et al*. (1993) 30h incubation	Phosphate buffer	3	20	30	36	1080
Martin *et al*. (1997)	Skim milk	2	45	64	1.8–6	256
Martin-Belloso *et al*. (1997)	Liquid egg	6	26	100	4	400
Matsumoto *et al*. (1991)	Phosphate buffer	5	40	80	14	1120
Qin *et al*. (1994)	Simulated milk	9	70	80	2	160

Table 15.1 Summary of PEF testing on *E. coli*.

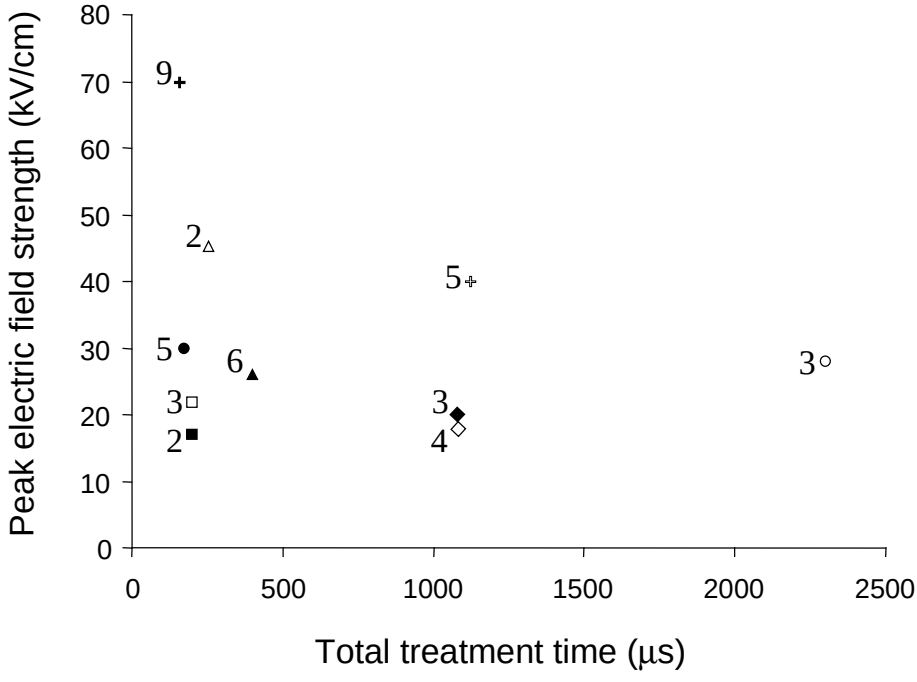


Figure 15.1 Summary of PEF treatment by various authors on *E. coli*:○ Dunn and Pearlman (1987), ● Evrendilek *et al*. (1999), □ Grahl *et al*. (1992), ■ Hamilton and Sale (1967), ◇ Hulsheger *et al*. (1983) 4 hour incubation, ◆ Hulsheger *et al*. (1983) 30 hour incubation, △ Martin *et al*. (1997), ▲ Martin-Barboso *et al*. (1997), ✣ Matsumoto *et al*. (1991), ✚ Qin *et al*. (1994).

The PEF results listed in Table 15.1 are plotted in Figure 15.1. The peak electric field strength is plotted against total treatment time. Each point represents results from a different author. The numbers beside each point signify the log reduction achieved. It can be seen from the plot that there is a wide spread of results. Increasing the peak electric field has a general trend toward higher log reduction. Increasing the total exposure time seems to have little effect. Apart from these general trends the results do not show high correlation. This wide variation in results may suggest that physiological differences in the bacteria themselves were causing the variation.

Two points are shown for Hulsheger *et al*. (1983). These represent *E. coli* harvested at two different stages of growth. Under nearly identical conditions, *E. coli* harvested after 4 hours of incubation showed a 4-log reduction, whereas *E. coli* harvested after 30 hours of incubation showed a 3-log reduction. This is not as significant a difference as the growth stage testing in this thesis. A possible reason for this is that after 30 hours of incubation the bacteria are still in an early stationary stage of growth. The testing in this thesis extends the incubation much further, with a corresponding decrease in high voltage effectiveness.

15.2 The protective mechanism of bacteria against strong electric fields

The physiological state of bacteria has a critical bearing on the effectiveness of high voltage treatment of water, as discussed in Chapter 14. Cells in an exponential (or active) state of growth are highly susceptible to electric field treatment. Cells in the

stationary growth phase have a high resistance to the electric field. Non-metabolising cells seem to have a protective mechanism that shields them from the effects of the electric field. Most organisms in naturally occurring water supplies are non-metabolising. Therefore, the death rate of bacteria in these water supplies would be much lower than initial laboratory tests have indicated. Further work is required to discover and isolate the physical or chemical mechanism in the bacterial cell that can be attributed to this electric field immunity or resistance. Better understanding of this protective mechanism is important so that new methods may be designed to overcome it.

Further investigation of this phenomenon could be undertaken on bacteria such as *E. coli*, which are well studied and documented. The following two predictions could then be made: 1) If the phenomenon is genetically-based, then it should be possible to select for mutants that are resistant to an electrical field. These mutants should be able to survive independent of the phase of growth when they are exposed to the field. 2) Changes within the phospholipid membrane are likely to play an important part in the resistance to an electrical field. It is clear that the fatty acids of bacterial membranes in the stationary phase undergo a transition from an unsaturated to a saturated state [Hood *et al*. 1986]. This transition may account for the observed resistance of post-exponential phase bacteria. To test this hypothesis, *E. coli* mutants that have different membrane or cell wall structures could be exposed to an electric field and their survival determined. In this way it may be possible to isolate any physical mechanism attributed to the electrical resistance.

It may also be useful to observe the formation of micropores in the cell membrane at different time intervals after exposure to a high electric field, building on work done by Chang (1992). Changes in the physical structure of the membrane in real time have not been observed at this level for the inactivation of cells and will give more information on the treatment mechanism. Equipment involved would be a scanning electron microscope (SEM) and/or an atomic force microscope (AFM).

15.3 The Effects of Current Density

Significant bactericidal effects have been reported using short applications ("impulses") of high electric current [Geren 1984]. Bacteria are electrocuted by the passage of electric current through the media. This is particularly effective when the rate of rise of current is very fast. By increasing the dI/dt, there is greater energy coupling at the higher frequencies, resulting in good current conduction through capacitive regions in the medium. This may include the cell membrane since this is mainly capacitive. Geren claims bactericidal effects are obtained when the current density through the media is increased above 50 mA/cm^2. Geren worked with current densities ranging from between 50 mA/cm^2 and 5 A/cm^2. The effective pulse duration used by the author was between 0.2 - 5 msec.

In most, if not all, published literature cited that relates to Pulsed Electric Field (PEF) treatment, the current densities are very high. This is one of the major limiting factors in the design of a practical device. The high current density contributes to a degradation of the electrodes and causes unwanted electrolysis problems. In the PEF treatment used by Hulsheger et al. (1981), the peak current density was 41 A/cm^2. The calculation for this is shown in Appendix C.

Schoenbach *et al*. (1997) applied PEF treatment to both tap water (1.9kΩ.cm) and nutrient broth (100Ω.cm). He found that for a given electric field strength, greater than a

critical value, the lysing rate was higher in the nutrient broth than in the lower conductivity tap water. According to Schoenbach, for a 60ns pulse, electric fields of 100 kV/cm are required to reduce the population of *E. coli* in tap water by one order of magnitude, compared to 70 kV/cm for a similar reduction in nutrient broth.

However, Schoenbach also found that the critical electric field strength required to start lysing *E. coli* with pulses of 60ns was the same for both tap water and nutrient broth (Ec = 40 kV/cm). From this he concluded that the viability of *E. coli* is mainly determined by the electric field, rather than by the current density in the suspension. In reality, the viability may be a combination of these factors.

For tap water (1.9kΩ/cm) with an applied electric field of 100 kV/cm, the current density is 52 A/cm^2. For the nutrient broth (100Ω.cm, 70 kV/cm) the current density is 700 A/cm^2. This is a large difference in current densities. It may be that the higher current density in the nutrient broth contributes to the inactivation of bacteria and thus a lower electric field strength is required to achieve a similar result.

15.3.1 Minimising Current Density at the Electrode Surface

One way of minimising the current density at the electrode surface is by the use of a Converged Electric Field (CEF) electrode chamber. This type of electrode chamber has been explained and used by Mizuno *et al*. (1990) and Matsumoto *et al*. (1991). The CEF electrode consists of two parallel plate disc electrodes spaced 20mm apart, with an insulating plate of thickness 10mm placed directly in between, as shown in Figure 15.2. The insulating disc has either one or a number of small holes through which the fluid passes through. The electric field is concentrated (converged) in these small holes and this is the region where cell lysing occurs.

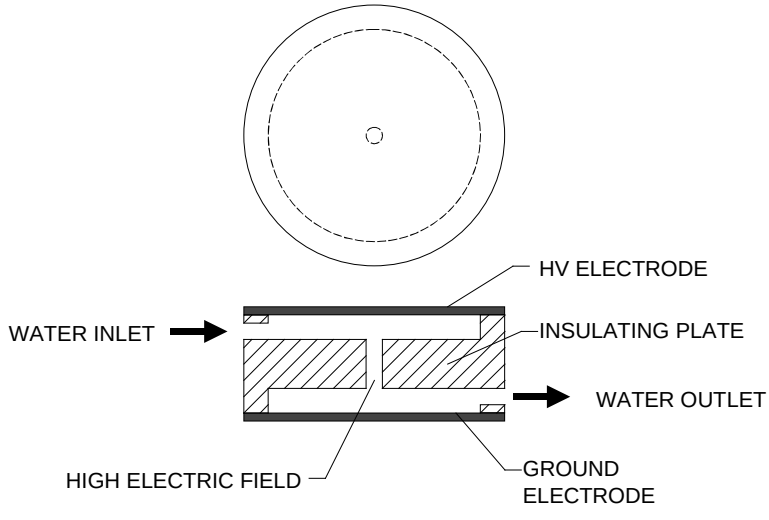


Figure 15.2 CEF electrode design

Matsumoto *et al*. (1991) found the CEF electrode chamber was more effective in lysing cells than a wire-cylinder electrode arrangement. They found that the survival ratio of *E. coli* suspended in a solution of conductivity 500 μS/cm could be reduced to 10^{-5} with a peak electric field strength of 40 kV/cm and energy density of 108 J/cm^3.

The CEF electrode has the advantage of low current density at the electrode surface. Another advantage is the added robustness to air bubbles and arcing between the electrodes, a major problem with the parallel plates of concentric cylinder electrode

systems. The concentric cylinder electrode design used for larger industrial sized flows, and described in Chapter 7, becomes less robust as the size and electrode surface area is increased. The electrodes become more prone to arcing or breakdown due to the high electric field and current density at the electrode surface. It may be more appropriate to use CEF type electrode chambers for large-scale applications since it would be much more robust to these effects.

15.4 Combination with Ultrasonic Disinfection

Ultrasonic energy may be transmitted through liquids and this has been shown to have a disinfection effect [Everett 1978, Scherba *et al*. 1991, Boucher 1980, Raso *et al*. 1998, Pagan *et al*. 1999, Suslick 1988]. The ultrasonic frequency employed is usually between 15-40 kHz. Destruction of microorganisms is believed to be due to the violent local changes in liquid velocity and pressure associated with the ultrasonic waves [Everett 1978].

In his US patent, Everett claimed a kill rate of around 99.5% on *E. coli* bacteria when using his ultrasonic disinfection device on a range of different flow rates. These tests were performed at an input power level of 100W, and an optimum flow rate of 10 litres per minute. At this flow rate Everett estimated the energy required per unit volume of liquid must be greater than 400 J/litre in order for the device to work effectively. To prevent ultrasonic energy being dissipated or absorbed by the solid walls of the pipe or channel, Everett forced the liquid through a nozzle to emit a fine spray. The spray is directed onto a vibrating ultrasonic surface at a direction perpendicular to the surface. It is this sudden contact with the ultrasonic surface, which enhances the disinfection effect.

Scherba *et al*. (1991) also found that ultrasound in the low-kilohertz frequency range had some efficacy in activating disease agents that may reside in water. They tested the germicidal efficacy of ultrasound on bacteria (*E. coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*), fungus (*Trichophyton mentagrophytes*), and viruses (*feline herpesvirus type 1* and *feline calicivirus*). Plastic bags containing the organism suspensions were suspended in an ultrasonic water bath. They found a significant reduction in viability for all tested organisms, except the *feline calicivirus*. They found the physical mechanism of inactivation appeared to be transient cavitation. Cavitation can affect a biological system by virtue of a localised temperature rise, mechanical stress, and/or free radical production. Since there was no significant difference of germicidal efficacy between gram negative and gram positive bacteria, they assume the inner (cytoplasmic) membrane has been disrupted rather than the outer cell wall.

The level of inactivation in Scherba's experiments was in the range 10-95%. This is a low inactivation rate when compared to other standard disinfection techniques. Thus the germicidal efficacy of ultrasound on its own is not sufficient to completely decontaminate common-use water facilities. However, it may be used in conjunction with other disinfection methods to increase the overall efficiency.

Ultrasound has been used in conjunction with heat and antibiotics to improve the efficiency of these treatments [Raso *et al*. 1998, Rediske *et al*. 1998]. The application of ultrasonic treatment makes bacteria more susceptible to these disinfection techniques. In light of this previous research, it would be reasonable to assume that ultrasonic energy could be combined with the high voltage technology in order to increase the overall effectiveness. Since both treatment methods disrupt the cytoplasmic membrane, they

may work together to be more effective. For example, if high voltage was applied first to initiate micropores in the membrane, ultrasonic energy may further disrupt the weakened membrane structure. Also, Allison *et al*. (1996) found that *E. coli* harvested in the stationary phase of growth were more susceptible to ultrasound than were those harvested in the exponential phase. This is opposite to the growth stage effect of the high voltage treatment. Thus the technologies may combine to cover all stages of bacterial growth.

Ultrasonic energy has one other advantage - it has been used to bring out dissolved air from solution. This would be advantageous for the high voltage technology if this could occur immediately prior to the electrode chamber. This may further reduce electrical breakdown and arcing between the electrodes due to air coming out of solution.

Research would be required to find the most effective combination of high voltage and ultrasonic technologies. If the ultrasound is applied before the high voltage, this may help to de-gas the water and make the device more robust. Applying ultrasound after the high voltage may improve disinfection performance. One further possibility would be to incorporate the ultrasonic device into the electrodes. One electrode could be attached to an ultrasonic device and made to vibrate in and out. This simultaneous combination of the vibrations and the high electric field may also enhance the biological effectiveness of the device.

A high voltage SMPS could be developed that can be used for both technologies. The existing SMPS operates at 17 kHz, as discussed in Chapters 12-14. This frequency is already in the ultrasonic range. Thus a universal supply could be used for both the high voltage and the ultrasound.

15.5 Combination with Chlorination

The application of small doses of chlorine immediately post high voltage treatment may be more effective than chlorine alone. The application of high voltage to cells causes the membranes to become permeable, thus making the cells more susceptible to the effects of chlorine. Haas and Aturaliye (1999a and 1999b) found *Giardia* cysts and *Cryptosporidium* oocysts became more susceptible to chlorination following the application of high voltage electroporation. This may apply to other organisms. Water pre-treated with high voltage may require much less chlorine for successful disinfection, thereby reducing chemical costs.

15.6 The Corrosive Effects of Deionised Water

Deionised or pure water is highly corrosive. Deionised water attacks any metal components that are in contact with it, because it is a universal solvent. This is why ultra-pure water is never found in natural water supplies. The extent to which a liquid will cause corrosion is measured by the Langelier index. A liquid with a negative Langelier index is corrosive whereas one with a positive index is non-corrosive. The Langelier index relates to the saturation levels of $CaCO_3$. The Langelier index is calculated for deionised water and Christchurch tap water in Appendix C. Deionised water has a Langelier index of –7.2, tap water an index of –0.6. This indicates deionised water will cause much more severe corrosion of metals than ordinary tap water. For this reason, any design of a high voltage water disinfection system must use a minimum

amount of metal components in the plumbing.

15.7 High Voltage Disinfection Technology in Air Conditioning Systems

The application of high voltage water treatment in recirculating systems was discussed in Chapter 13. It was found that for a recirculating system the disinfection rate was not critical to the overall performance in reducing the levels of bacteria in the system. The high voltage technology was well suited to this sort of application, including air conditioning systems.

However, when using a deionising resin in conjunction with the high voltage, the corrosive effects of deionised water must be considered. New Zealand standards for air conditioning systems specify the maximum amount of metal lost to corrosion as <5 mil (5/1000 inch) per year. This is normally achieved in air conditioning systems by the regular addition of a chemical corrosion inhibitor. These inhibitors would not be compatible with a deionising resin. They would most likely be removed from the system by the resin, and also cause fouling of the resin bed.

The high voltage treatment system is a point-of-use (POU) device, and has no residual disinfection. In other words, it may disinfect a flow of water at one section of the air conditioning system, but has no effect on the growth of bacteria in other parts of the system. Residual disinfection (such as chemical biocides) adds a disinfection property to the water itself, and the water carries the disinfectant to all parts of the system.

There are two main considerations for bacteria growth in air conditioning systems, general bacteria levels in the water, and bacteria growth on the pipes and walls of the system. Bacteria readily adhere to pipes, and when conditions are right multiply very quickly and form a biofilm or slime on the pipe surface. Some types of bacteria obtain nutrients from the pipe surface itself and cause corrosion of the pipes. Since conditions in the air conditioning system can be ideal for bacteria (20-35°C), growth can occur very quickly. The system becomes blocked up with bacteria slime and heat exchangers can become inefficient. Two of the main problem areas of biofilms are the heat exchanger (warm temperatures) and cooling tower baffles. To prevent the build up of biofilms on these surfaces, existing air conditioning systems are generally treated with residual biocides on a weekly basis. A POU device cannot prevent the growth of these biofilms within the system. Even if the entire system was cleaned out and sterile, the associated cooling tower is still open to the atmosphere and susceptible to biological contamination.

A POU device may be installed in these types of systems if it is used in conjunction with chemical treatment. However, these types of chemicals cannot be used in a deionised system as they will also be removed by the resin. The deionising resin will also be fouled and damaged by these chemicals. Therefore, for the high voltage treatment method to be used in air conditioning systems, the deionising resin must first be eliminated. This may be achieved by the use of a SMPS, however further development is required before this becomes a reality. The high flow rates and large volume of typical air conditioning systems mean the existing SMPS needs to be scaled-up in size. The continual evaporation of water means the system conductivity increases over time. The SMPS needs to treat water of higher conductivities. If these problems can be solved and a suitable SMPS designed, perhaps this treatment method would be useful in conjunction with a chemical disinfectant.

Chapter 16

CONCLUSIONS

Applications for high voltage disinfection and treatment of water have been presented in this thesis. A demonstration unit with a one litre per minute (1 LPM) flow rate was constructed and tested. This device uses an electric field strength of 30 kV/cm at 50 Hz. The initial demonstration model was shown to work effectively both electrically and biologically. Initial tests on the bacteria *S. marcescens* showed promise for the technology. A three-log reduction in viability was obtained at an exposure time of 17 msec. Many researchers using pulsed electric fields have cited even higher reductions in viability of a wide range of bacteria. Thus, it was logical to assume from these results that the performance of the initial demonstration model could be greatly improved given a better design.

The initial 1 LPM demonstration model was redesigned to allow for commercialisation and mass production. Necessary changes were made to the high voltage supply, air trap and electrode chamber. These design changes enabled the respective parts to be mass-produced and also lower costs of production. The commercial prototype was tested and the air trap / electrode chamber combination was found to be more robust in operation. The commercial prototype was also tested on *S. marcescens* and results were comparable to that of the initial development model, although slightly lower.

The high voltage treatment technology was scaled up to higher flow rates. 10 LPM and 33 LPM demonstration devices were constructed. The larger flow rates of these devices required a design change of the electrode chamber. Pairs of concentric cylinder electrodes were used instead of parallel flat plates. However, it was found that these larger flow rate devices were less robust than the 1 LPM device. The high voltage transformer for the 10 LPM device had a higher rating than that of the 1 LPM. A soft-start circuit needed to be incorporated into the device to prevent inrush current problems on start-up. Also, the larger surface area of the electrode chamber exacerbates arcing and instability problems. Hence, a diverter valve and drain sequence was required to increase the robustness upon start-up. When the device has been sitting idle with no water flow, an increasing amount of ions are dissociated into the water by the electrodes, thereby increasing the conductivity of the water around this region. This increased conductivity water must be flushed out from the vicinity of the electrodes before the high voltage is applied.

The added complexity of the higher flow rate devices can best be handled by a microcontroller. The incorporation of a microcontroller into the 10 LPM device enabled accurate monitoring of the high voltage primary current, control of the arc and resin alarms, and an automatic reset function. It also allows for an intelligent drain sequence and flushing procedure. Use of this feature ensures the minimum amount of water is wasted by the drain sequence. The microcontroller also has a serial RS232 port to allow

the download of system performance and alarm history either locally or remotely by use of a modem.

The higher flow rate devices (10 LPM and 33 LPM) were tested on *S. marcescens*. The performance of these devices in reducing the viability of bacteria was expected to match or be even higher than that of the 1 LPM device. This was because of the expected improvements made by the concentric cylinder electrode system. These electrodes have no side edge effects and regions where water may bypass the electric field. Contrary to this expectation however, results showed a significant decline in effectiveness on *S. marcescens*, even at similar electric field strengths (30 kV/cm) and higher exposure times. Possible reasons for this finding were examined and experimentally tested. There was no obvious explanation for this difference in results between the 1 LPM and the higher flow devices. This may present a problem in the scaling up of the high voltage technology.

A high voltage switch-mode power supply (SMPS) was designed and constructed. This supply provides a bipolar square waveform at 3 kV, 17 kHz, and 400 VA. The SMPS has many advantages over a 50 Hz waveform. It enables the exposure time to be significantly reduced since the duration of areas of low electric field strength has also been reduced. This reduction in exposure time enables the operating energy requirements to also be reduced. The energy required to treat deionised water may be reduced by over 90%. This reduction in energy requirements enables water of higher conductivity to be treated. The reliance on the deionising resin may be reduced or even eliminated. The demonstration SMPS was tested on *S. marcescens* and *E. coli*. It was found that the viability of both organisms could be reduced by 90 - 99% by one application with an exposure time of only 1.3 msec. The effectiveness of the SMPS is thus not as great as the 1 LPM device, but multiple applications of this may reduce the viability even further.

Use of a high voltage SMPS in recirculating systems has been examined. The bacteria population in the overall system may be reduced by continually treating a side stream of the water and feeding it back into the system. The reduction in bacteria numbers may be increased by either increasing the recirculation time or the side stream flow rate. According to a theoretical model for the bacteria reduction, a side stream treatment that reduces bacteria numbers by 99% is just as effective over time as one that reduces the numbers by 100%. It is also found that increasing the side stream flow rate can reduce numbers more effectively. Therefore, a recirculating treatment system may be operated at higher flow rates at the expense of a lower immediate bacteria reduction, and still be more effective in reducing overall numbers of bacteria within the system. Also, the effectiveness of the SMPS is high enough to be considered for these applications. The theoretical model for recirculating systems was verified by experimental testing using *E. coli*. The experimental results closely match those of the theoretical model. An overall reduction in the viability of *E. coli* during some of these tests was greater than 4-log after a recirculation time of 15 minutes. This reduction in viability was greater than any previous testing on the 1 LPM, 10 LPM and 33 LPM devices.

The effect of cell growth phase on the treatment effectiveness was examined using *S. marcescens* and *E. coli*. It was found that the physiological state of the bacteria had a great effect on the effectiveness of the high voltage treatment device. The effect of growth phase outweighs all other effects, such as field strength and exposure time. Bacteria harvested in the early logarithmic stage of growth were most susceptible to the electric field treatment. Bacteria harvested in the late stationary phase were much more resistant.

Results from most of the bacteria testing in this thesis came from experiments on cells harvested in the early stationary phase of growth. This is the standard method for cultivation of bacteria and the testing of treatment devices. Since the state of most naturally occurring bacteria in water supplies is that of low growth, this method of testing does not give an accurate measure of the effectiveness of the device on naturally occurring water supplies. The test results in a laboratory situation will be much higher than those in a natural situation for which the device is designed.

Further research is required to better understand the mechanism behind the resistance of bacteria to the electric field treatment before the device can be effectively used in commercial applications.

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Appendix A

PLAN FOR COMMERCIAL PRODUCTION OF 1 LPM WATERISER FOR MALAYSIAN MARKETS

A1. Introduction

This Appendix discusses the commercial application of the Wateriser technology to the area of domestic drinking water supplies. The process of initiating a commercial production run of Wateriser units for the domestic market is documented. The production has taken place at a factory in Kuala Lumpur, Malaysia. Prospective markets for this device are household point-of-use (POU) drinking water applications worldwide.

A2. Project Objectives

A2.1 Marketing Objective

The objective of this project is to develop the Wateriser water purification system into a range of high value hot and cold pure water dispensers for domestic and commercial markets.

The domestic Wateriser has been initially aimed at the local Malaysian market. The first few thousand Wateriser units will be marketed locally for two reasons. Firstly the manufacturing company, Abric Berhad, already has a marketing infrastructure in Kuala Lumpur through a number of electrical appliance stores. The second reason is that of servicing. The first few Wateriser units can be monitored more closely in case of unforseen technical problems that may occur once the product is being used by consumers. It is also more convenient to set up a replacement and regeneration service for exhausted deionising resin cartridges (see Section 3).

The demands of the Malaysian market may differ from that of other overseas consumers. The Malaysian requirements were assessed by local knowledge and by a survey of typical consumers. The requirements are summarised as follows:

- Attractive presentation (On public display in household).
- Simple to operate.
- Easy to service.
- Clear, visually pleasing water output.
- Good taste.
- Water safe to drink.
- Hot and cold water options are an advantage.

To achieve both aesthetic objectives (taste and colour), a carbon pre-filter is necessary to remove particulate matter and reduce organic and taste affecting compounds. The pre-filter also needs to contain carbon in order to remove free chlorine in the water and protect the deionising resin from physical damage (see Section 3).

For the initial Wateriser model it was decided not to incorporate hot and cold water options. These would increase the complexity, cost, size and weight of such a device. A later model Wateriser may incorporate these features at a later stage.

The operation, servicing and water safety requirements are all inherent in the initial development model of the Wateriser (Chapter 4). These requirements will however need to be taken into consideration in the design of the production model. The above aspects will also need to be tested and certified before production can begin.

A2.2 Product Development Strategy

The strategy is to introduce the Wateriser technology to the market-place in three phases:

1. Refine the development model Wateriser into an ergonomically designed counter-top home water purifier that operates at normal room temperature.
2. Develop and introduce a commercial hot and cold water purifier targeted specifically at offices, using the present Wateriser system.
3. Introduce a counter-top hot and cold water purifier.

This report only covers the first phase of the strategy, the development of a counter-top home water purifier.

A2.3 Market Research

Other drinking water systems that are currently available in the Malaysian market are listed in Table A1.

Water treatment system	Relative Cost
Hot and cold water dispenser, pipe-in, free standing, counter-top or wall hung, using filters.	1,400 - 2,500
Hot and cold water dispensers, with bottle, free standing, counter-top or wall hung, using filters.	1,200 - 2,500
Hot and cold water dispensers, free-standing using reverse osmosis purification system.	?
Wall mounted water purifiers using ultra-violet radiation.	2,500 - 3,500
Water distillers.	1,200 - 2,500

Table A1 Domestic and commercial water treatment devices and their relative costs in Malaysian dollars (Ringgit).

The Wateriser will be positioned as a high value-for-money premium technology alternative to all the above systems.

A2.4 Product and Component Sourcing

Where possible, components are to be sourced or manufactured locally. This is critical to keeping production costs to a minimum. Component specifications are to be finalised and vendor selection done. Any parts that require manufacturing will be initially outsourced. When production volume and experience is stabilised, all plastic injection moulding will be done by Abric Manufacturing. Abric will assemble components and test the completed Wateriser units. They will be responsible for product distribution, servicing and quality control procedures.

A2.5 Costing Objective

The aim of this project is to keep the total cost of production to RM$800.

A3. Project Overview

The project of setting up a production line for the domestic, counter-top Wateriser can be broken into the following sections.

- Component Design
  - Sourcing of suppliers
  - Manufacturers of non-existing components
  - Component documentation
- Production Line Details
  - Assembly processes
  - Documentation
  - Staffing resources
  - Training
  - Physical requirements
  - Floor plan
  - Tools and equipment required
  - Quality testing
- Quality Control Testing and Certification
  - QC test equipment
  - QC procedures
  - Independent certification
  - Government standards for drinking water (SIRIM approval)
  - Biological killing effectiveness (Independent laboratory)

The procedure is also outlined in the flow chart in Figure A1.

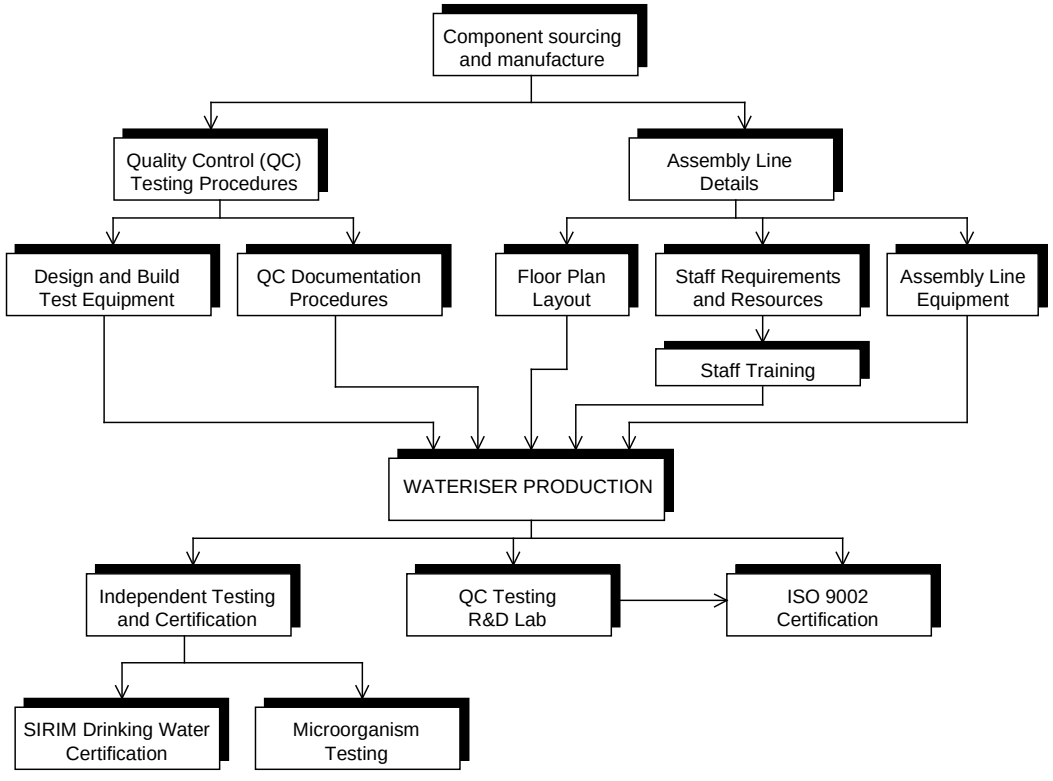

Figure A1 Wateriser production flow chart

A4. Component Design

A block diagram of the Wateriser can be seen in Figure A2.

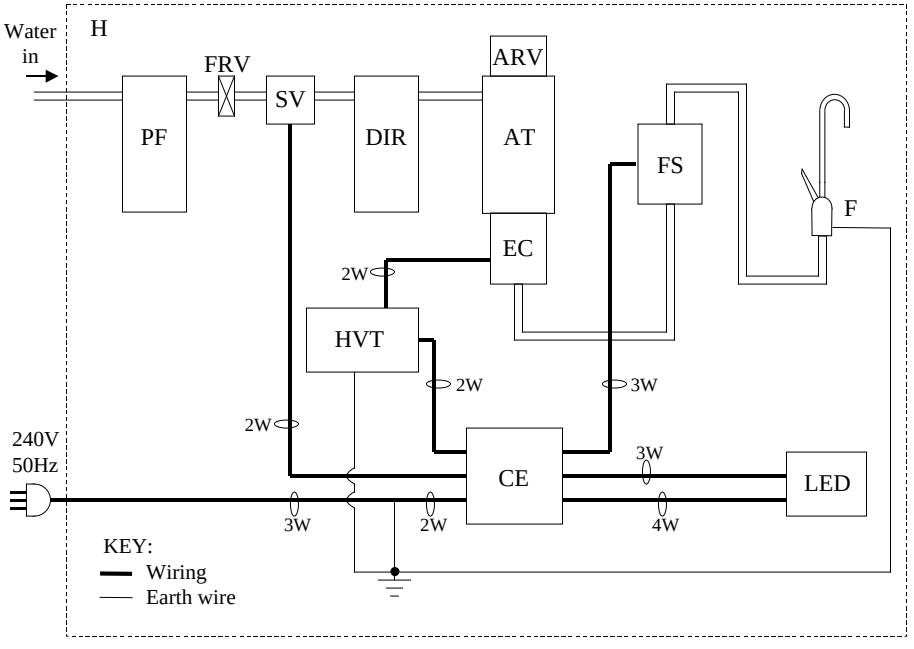

Figure A2 Block diagram of Wateriser process

The Wateriser has a water inlet and 240V mains power supply. The output from the Wateriser is through the faucet (F) and display panel (LED) that is mounted on the housing.

The main components of the Wateriser are:

ARV	Air Release Valve
AT	Air Trap
CE	Control Electronics
DIR	Deionising Resin
EC	Electrode Chamber
F	Faucet
FRV	Flow Restriction Valve
FS	Flow Switch
H	Housing
HVT	High Voltage Transformer
LED	Display Panel
P	Plumbing
PF	Pre-Filter
SV	Solenoid Valve

The design of each of these components is briefly described in the following sections.

A4.1 Component Function

The Wateriser may be broken into 14 separate components. The general function and description of these are described in this section.

A4.1.1 Air Release Valve (ARV)

An automatic air release float valve mounted at the top of the air trap. The ARV removes any air that builds up in the air trap.

A4.1.2 Air Trap (AT)

The purpose of the air trap is to remove any air bubbles present in the water, originating from either the supply or introduced by the replacement of the pre-filter or deionising cartridges. Air bubbles are undesirable and detrimental to the electrodes as they cause flashover and arcing. The air trap consists of a cylinder of sufficient cross-sectional area that slows the velocity of the water such that air bubbles float to the top where they are released from the trap by the air release valve.

A4.1.3 Control Electronics (CE)

The control electronics include a +15V supply, switched triac control of the high voltage transformer and solenoid valve, high voltage transformer current sensing via a current transformer, control logic, and safety alarm features.

A4.1.4 Deionising Resin (DIR)

The deionising resin consists of a mixed bed ion exchange resin within a refillable plastic cartridge for regeneration purposes. The resin removes both cations and anions

that are present in the source water, reducing the contamination level, and thus conductivity to less than 1 μS/cm. This conductivity level is required to enable the Wateriser to run at low power levels of 40-60W.

A4.1.5 Electrode Chamber (EC)

The electrode chamber consists of two circular flat electrodes embedded in plastic with a water channel that flows between them. The chamber is injection moulded, and the water channel is 12mm wide and 1.5mm deep. The electrodes are approximately 16mm diameter and are machined out of stainless steel, grade 316T.

A4.1.6 Faucet (F)

A tall chromed faucet is mounted on the Wateriser housing. The height is required to enable 1.5-litre water bottles to be filled underneath.

A4.1.7 Flow Restriction Valve / Washer (FRV)

The biological performance of the Wateriser is dependant upon the flow rate of the water. The flow rate for this electrode design must be below one litre per minute to ensure effective bacteria deactivation. The flow restriction washer consists of a rubber orifice mounted in a machined brass insert. The rubber orifice constricts the flow of water according to the input pressure. The rubber orifice deforms under different input water pressures to provide a roughly constant flow rate.

A4.1.8 Flow Switch (FS)

When the faucet is opened there is an initial flow of water due to the small inherent pressure in the system. The water flow causes a stainless steel slug to move up through a transparent tube where it interrupts a light beam. In this way the flow switch is activated when the water begins to flow, and the Wateriser activates the disinfection.

A4.1.9 Housing (H)

The Wateriser housing is constructed from fibreglass, and covers the Wateriser components. The front display panel is mounted for easy access and viewing. There is a removable panel on the back of the housing that allows access to the pre-filter and deionising resin cartridges.

A4.1.10 High Voltage Transformer (HVT)

This is a 50 Hz, 240:6000V step-up transformer that provides the high voltage required at the electrodes. The transformer is rated at 150 VA.

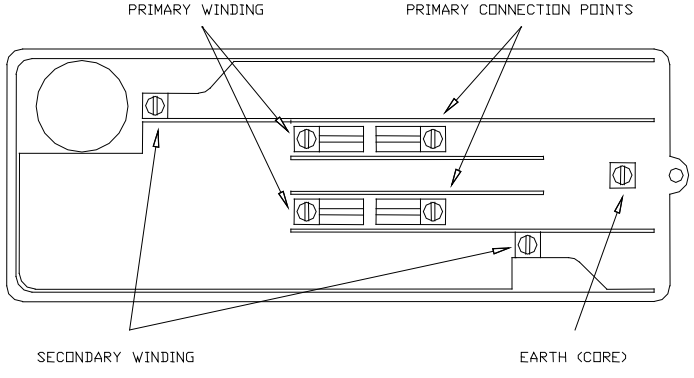

Figure A3 High Voltage Transformer

A4.1.11 LED Display and Front Panel (LED)

The front panel consists of the ELCB on/off switches, a red power LED, a green resin LED, and the reset switch. The reset switch is necessary to reset alarms and upon installation to fill the device with water.

A4.1.12 Plumbing (P)

The plumbing provides the water path from the water mains, through the Wateriser and electrodes, and to the faucet for potable use. All plumbing pieces must be constructed from non-toxic or food grade material.

A4.1.13 Pre-Filter (PF)

The pre-filter removes solid material and sediment from the input water supply. It is a 5 μm carbon block filter. The carbon is required to remove residual chlorine from the water before it reaches the deionising resin where it could cause physical damage to the resin beads. The sediment needs to be removed for aesthetic and health reasons, and also to prevent blockage of the solenoid valve and flow restriction washers.

A4.1.14 Solenoid Valve (SV)

The solenoid valve turns the input water flow on and off. It is required for the security aspects of the device. If a fault occurs, or the deionising resin requires replacement the solenoid valve will shut off the flow of water so the consumer is protected from obtaining untreated water.

A5. Production Line Details

A5.1 Assembly Process

The assembly process may be broken up into seven distinct sections as explained by the process flow diagram in Figure A4.

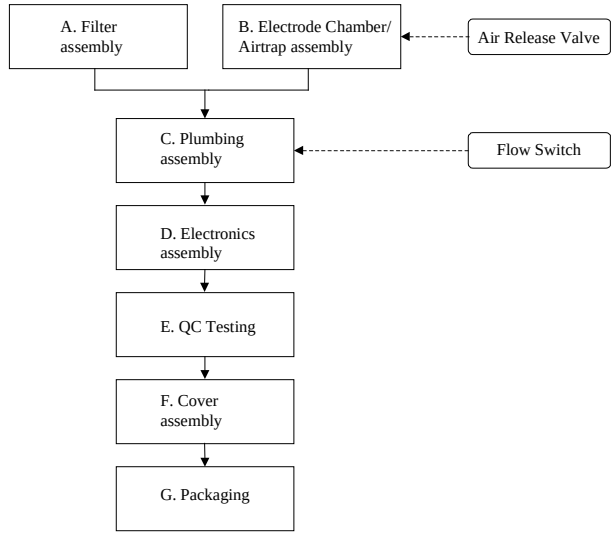

Figure A4 Process flow diagram

A5.2 Physical Requirements

A5.2.1 Floor Plan

A room and floor space in the existing Abric factory has been allocated as the Wateriser assembly area. The total floor space is 50' x 50'. A proposed floor plan for the assembly of the Wateriser is shown in Figure A5. The key for the assembly stages is as listed in Section A5.2.2. Two existing offices in the assembly area have been allocated for the product manager and office staff. These offices are to be equipped with desks, stationary equipment and computers.

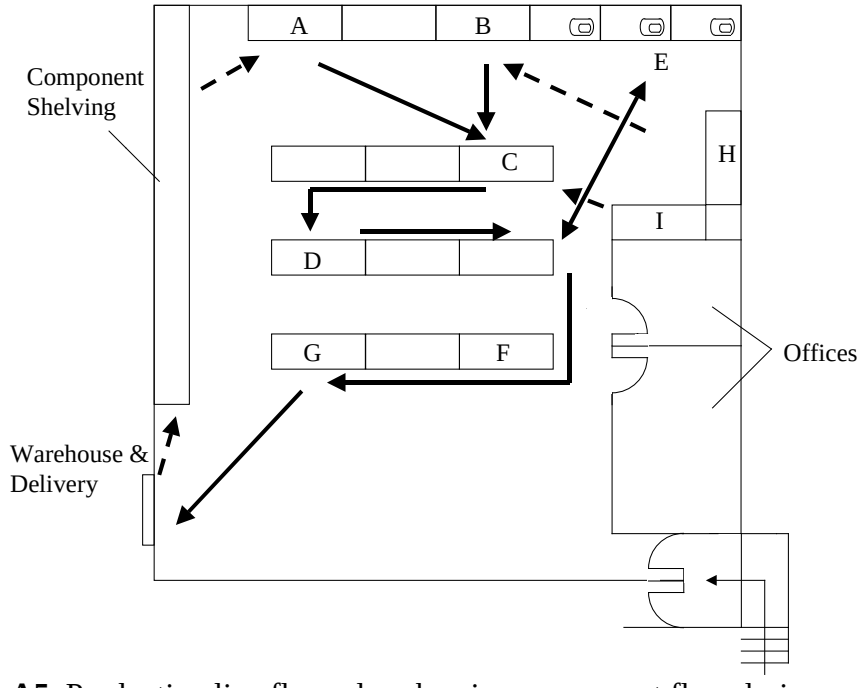

Figure A5 Production line floor plan showing component flow during assembly.

Nine stainless steel covered benches, 8' wide and 3' deep, were constructed for the assembly area. The size of each bench allows adequate space for two assembly workers. There is room for a further five similar benches to allow for expansion. The positions for these additional benches are also indicated on the floor plan. In one corner of the room is a 6' x 3' stainless steel covered bench, equipped with stainless steel sink and water connections. The purpose of this bench is for quality and functional testing of the assembled Wateriser units. Again, there is room for a further two similar quality testing benches in the spaces indicated.

Two of the 8' benches are positioned along the wall adjacent to the 6' quality testing bench. These are for the assembly of the AT/EC components. Some use of solvents and silicone sealant may be used in this process, so these benches are equipped with extractor fans mounted through the outside wall.

Another 8' bench is situated next to the product manager's office. This bench is used for assembly of the flow switch components.

The remaining six 8' benches are arranged in the middle of the floor in three rows of two benches each. The first row is used for the filter and plumbing assembly process. The second row is for the electrical wiring and connections. The third row is for final

assembly of cover and packaging. Each of these three rows can be expanded as required production levels increase.

On the wall opposite the two offices are wall mounted storage shelves. These shelves are to hold the supply of Wateriser components required for assembly. These shelves can be replenished from the store on a daily basis.

Each bench is equipped with mains power. The power leads for the benches in the middle of the floor are brought down from the ceiling. The two AT/EC assembly benches are equipped with a supply of compressed air. The QC test bench is also supplied with two water connections. One mains water connection and the other high pressure connection provided by a compressor mounted beneath the sink bench.

A5.2.2 Assembly line tools

Each section of the assembly line process will be equipped with the necessary tools required for effective assembly, listed as follows.

A. Filter assembly
    - A.1 Thread tape
    - A.2 Ring/open ended spanner set
    - A.3 Socket drive
B. Electrode chamber / air trap assembly
    - B.1 Compressed air supply
    - B.2 Burr remover (scraper)
    - B.3 1/4" NPT tap set.
    - B.4 Open ended spanner.
    - B.5 PVC/ABS plastic welding cement.
    - B.6 Thread tape.
    - B.7 Vice.
C. Plumbing assembly
    - C.1 Thread tape.
    - C.2 Ring/open ended spanners.
    - C.3 Screw driver set.
    - C.4 Nut driver.
D. Electronics assembly
    - D.1 Screwdriver set.
    - D.2 Nut driver.
    - D.3 M4 open ended spanner for electrodes.
E. QC Testing
F. Cover assembly
    - F.1 Nut driver.
    - F.2 Open ended spanner set.
G. Packaging
H. Air release valve
    - H.1 Vice.
    - H.2 Pipe wrench.
I. Flow switch assembly
    - I.1 Slug stopper tool.
    - I.2 Plastic cement.
    - I.3 Hot glue gun + glue sticks.
    - I.4 Tubing cutter.

A6. Quality Control Testing and Certification

A6.1 Wateriser Assembly QC Test Procedure

This QC test involves an electrical and water functionality test of the partially assembled Wateriser unit. This test is to be run after all plumbing and electrical components are in place, but before the outer housing has been attached. A description of the test procedure is described as follows:

E1. **Connect Plumbing**
- 1.1. Screw in 1/4" BSP inlet connection to pre-filter (PF).
- 1.2. Connect flow switch (FS) output to test faucet.
- 1.3. Screw in test filter housings (PF and DIR housings).

E2. **Connect Electrical**
- 2.1. Plug in LED display connector from test board.
- 2.2. Plug in 240V power socket.

E3. **Water Pressure Test**
- 3.1. Set water pressure at 150 p.s.i. Turn on water valve. Check for leaks in the plumbing up to the solenoid valve.
- 3.2. Activate RESET switch. Wateriser pressurises up to full pressure (150 p.s.i.).
- 3.3. Turn off input water valve.
- 3.4. Check for water leaks throughout plumbing. Pressure reading does not drop.

E4. **Flow Test**
- 4.1. Turn on input water valve.
- 4.2. Operate the faucet until a steady flow of water is coming from the faucet. Measure the flow rate.
- 4.3. Measure the water conductivity. When the conductivity is below 1uS/cm then continue with the following HV test.

E5. **High Voltage Test**
- 5.1. Turn off the RESET switch.
- 5.2. The High Voltage should be operating (Operator should NOT touch equipment while it is live). The flow of water should remain constant. Measure primary current. Current should be in the range 200-800 mA rms. Record current on inspection sheet.
- 5.3. Turn off faucet and then back on. Repeat several times to ensure unit is functional and no alarms occur. Primary current should be stable.

E6. **Drain Water and Reassemble Wateriser**
- 6.1. Turn off water supply.
- 6.2. Turn on RESET switch and operate faucet to release water pressure.
- 6.3. Disconnect electrical power.
- 6.4. Remove LED display connection.
- 6.5. Undo and remove PF and DIR housings.
- 6.6. Disconnect plumbing at the bottom of air trap (AT) or flow switch (FS). Drain the Wateriser of all water.
- 6.7. Remove the input plumbing connection from pre-filter (PF).
- 6.8. Reconnect the plumbing at the bottom of AT or FS.

E7. **QC Inspection Sheet**
- 7.1. Ensure QC inspection sheet is completed and filed.

A flow chart for this process is shown in Figure A6.

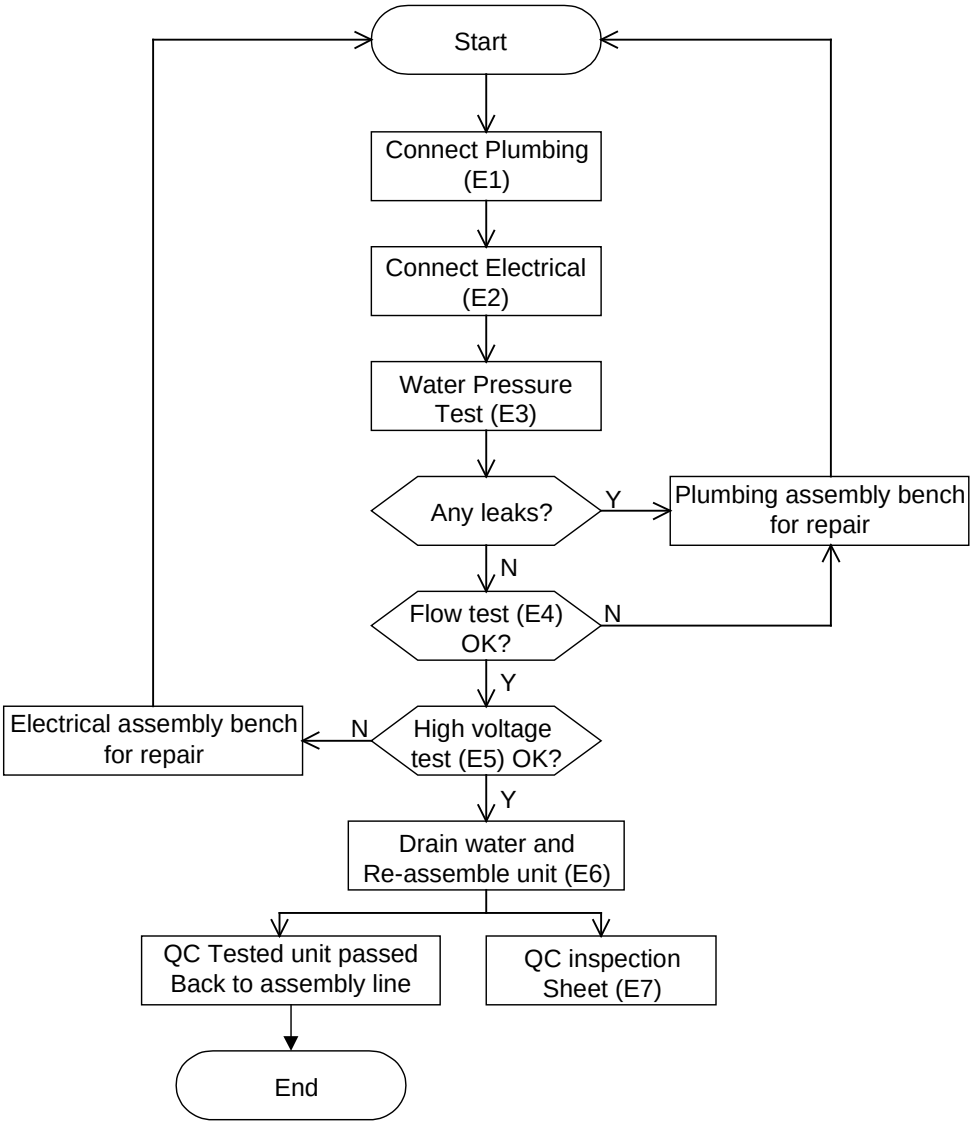

Figure A6 Wateriser assembly quality control test procedure

A6.2 QC Testing R&D Laboratory

Further QC testing will also be undertaken in the R&D laboratory. A sample of output Wateriser units will be installed and tested to ensure operation is complete. Also, a random sample of incoming components will be tested to ensure they meet the QC specifications. Any defective components or batch of components may need to be readdressed to the manufacturer, and the problem rectified.

A6.3 ISO 9002 Certification

Once the QC procedures are in place, application should be made for ISO 9002 certification. It is important that internationally recognised quality assurance procedures are set in place to ensure output quality. This is important for the Wateriser since (a)

consumer safety needs to be ensured in light of the potentially harmful electrical connections present in the device, (b) consumers need confidence in the quality of the output product (ie. water), and (c) the device will potentially be exported world-wide.

A7. Wateriser Component Costing

Following the development of the Wateriser and the location of suitable component manufacturers within Malaysia and abroad, the finalised costing summary of the necessary components is shown in Table A2.

Description	**Supplier**	**Qty**	**Unit price**	**Total**
10" filter housing, 1/4" BSP thread	Ecspec, KL	2	75.00	150.00
240VAC Solenoid, 1/8" BSP, NO	Baccara, Israel	1	35.00	35.00
Plumbing - misc	WT Hose, KL			27.65
Stainless Steel slug (flow switch)	Topaire, KL	1	2.00	2.00
Faucet	Touch-flo, USA	1	33.00	33.00
Air release valve	Giacomini, Italy	1	20.00	20.00
Airtrap / Electrode assembly	Abric / Topaire, KL	1	20.00	20.00
4.75 kV transformer	Siet, Italy	1	196.47	196.47
Electronic boards	Micontech, KL			108.00
Labels	Lasertech, KL	2	4.00	8.00
10" carbon filter	Ecspec	1	22.00	22.00
10" deionising cartridge (refillable)	Systematix, USA	1	39.00	39.00
1L deionising resin	Deltaflex, KL	1	20.00	20.00
Steel filter bracket	Topaire, KL	2	3.00	6.00
Fibreglass housing	SL Polymer, Malaysia	1	130.00	130.00
Misc. Nuts, bolts & washers	Asia-tech, KL			14.52
Flow restriction washer	Mariac, Australia	1	7.15	7.15
TOTAL				$838.79

Table A2 Costing for Wateriser components. Costs in Malaysian currency (Ringitt). Costing applicable at May, 1998. Note: This costing does not take into account assembly of the device.

Appendix B

NEW ZEALAND STANDARDS RELATING TO SMALL SCALE WATER TREATMENT DEVICES

B1.

AS/NZS 4348 : 1995 "Water supply - Domestic type water treatment appliances - Performance requirements"

Applicable points:

1.5.1 "The design of the appliance shall be such that failure of the consumable shall be automatically obvious to the user through flow cessation or rapid reduction in flow, alarm or simple explicit instructions for determining the need for servicing units to eliminate the possibility of a non-functioning appliance being relied upon by the consumer."

1.5.2 Materials to be food grade to recognised standards.

2.2 (b) "Those appliances claiming to be microbiological treatment appliances shall demonstrate that the reduction requirements for bacteria, viruses and protozoa as specified in Table A1, Appendix A, can be continually met or exceeded by both production units. Be aware that bacteria, viruses and protozoa shall all be removed as specified to qualify an appliance as a microbiological treatment appliance."

Table A1 Schedule B

Bacteria:	Klebsiella terrigena (ATCC-33257)	99.999%
Virus:	Poliovirus I (LSc) (ATCC-VR-59)	99.99%
	Rotavirus (WA or SA-11) (ATCC-VR-899 or VR-2018)	99.99%
Protozoa:	Giardia lamblia	99.9%

Comments:

- The use of a solenoid valve to stop the water flow when disinfection is not taking place is essential. This also provides a huge advantage over competitive treatments (UV) that cannot provide such a strong guarantee.
- All production materials are to be checked for food grade.
- Electrodes alone may not achieve the bacteria reduction requirement. With a combination of filter/DI/electrodes this specification may be achieved. Again, testing with the appropriate organism should first be undertaken.
- The virus reduction ability of the technology is an unknown. The NZ Drinking Water Guidelines state both activated carbon and ion exchange devices as being moderately efficient at virus removal. Perhaps with the combination of these units the virus reduction requirement may be met even without considering the electrodes?

- The effectiveness on Giardia has already been proven. With the integrated device (filter/DI/electrodes) this requirement can easily be achieved, even for Cryptosporidium.

B2.

NZS 3497 : 1998 "Drinking water treatment units - Plumbing requirements"

Applicable points:

4.2 Design categories applicable to our technology:

(a) Bactericidal/microbiological
(b) Mechanical filtration
(c) Taste and odour reduction
(d) Chemical or mineral reduction

If any of these categories are to be claimed by the technology, device needs to comply with the appropriate section in AS/NZS 4348.

4.4 Solenoid is required to warn the user when the device is not performing its claimed functions. (Alternatively a flashing light or audible alarm may be used).

4.5 Any DI resin media should not be visible in the output water.

4.6 Flow restriction is necessary to prevent loss in performance. Alternatively the max/min recommended flow rate must be stated in product specifications.

4.8 When appliance is connected to a permanent hydrostatic pressure, a backflow prevention device is necessary (AS/NZS 3500.1.2 and AS/NZS 2845.1).

5.2 Appliance to withstand a hydrostatic water pressure test at 2070 kPa for 15min.

5.3 Endurance pressure test. 100,000 pressure cycles (0-1034 kPa) [AS 3707].

5.4 Minimum burst pressure of 2.76 MPa or 4 x nominated maximum working pressure.

Comments:

- The addition of a flow restriction valve on the Electropure e-10 is important, not only to meet this standard but also to prevent transient water flows and preserve the life-time of the deionising resin.
- The backflow preventer may be the solenoid valve. In this case it should be installed as close to the water inlet as possible.
- Again, the solenoid valve is important not only to ensure fail-safe operation, but also to prevent backflow problems.
- Refilling or regeneration of the DI cartridges should ensure no possibility of resin contamination at the DI output.
- The carbon pre-filter and deionising resin are integral parts of the technology. The filter performs categories (b) and (c), the DI satisfies (d) and the filter/DI and electrodes satisfy (a). The technology needs to incorporate all three of these components.
- Research and testing on the pressure rating of the devices needs to be undertaken and also incorporated into the design.

Appendix C

MISCELLANEOUS CALCULATIONS

C1. Calculation of Current Densities used by Hulsheger et al. (1981)

Typical parameters in these experiments were:
Pulse number, *n=10*
Field strength, *E = 12 kV/cm*
Capacitance, *C = 1 μF*
Solution resistivity = *600 Ωcm*
Solution conductivity, *κ = 1,670 μS/cm*

For a high voltage capacitive discharge, the electric field across the solution is given by

$$E(t) = \frac{U_o}{d} e^{-t/\tau}$$

and the current by

$$I(t) = \frac{U_o}{R_L d} e^{-t/\tau}$$

where τ = discharge time constant = RC = 36 μs
d = distance between electrodes = 0.5 cm
R_L = load resistance between electrodes = τ / 1 μF = 36 Ω

Given that the electrode surface area is 8 cm^2 and the peak voltage and current occurs at time t=0, the peak current density is

$$J_p = \frac{I_p}{A} = \frac{333A}{8cm^2} = 41 \text{ A/cm}^2$$

This current density is for pulses of relatively low field strength (12 kV/cm). Most PEF treatment systems use three or four times this field strength, thereby increasing the current density three or four times for treatment of fluid with a similar conductivity.

C2. Calculation of Langelier Index for Different Water Types

C2.1 Deionised water

Parameters (from SIRIM laboratory test report - Malaysia)

TDS	< 1	mg/L			
Conductivity	<1	μS/cm			
$[Ca^{+2}]$	< 0.06	mg/L	$=1.5\times10^{-6}$	mol/L	(40.0 g/mol)
$[HCO_3^-]$	< 0.1	mg/L	$=1.6\times10^{-6}$	mol/L	(60.8 g/mol)
pH	6.5				

The ionic strength, μ, can be estimated from the formula [Langelier]:

$$\mu = (2.5\times10^{-5})\times TDS$$
$$= 2.5\times10^{-5}$$

The activity coefficient for HCO_3^- can be calculated from:

$$\log\gamma_{HCO_3^-} = -\frac{0.5(z_i)^2\sqrt{\mu}}{1+\sqrt{\mu}}$$

$$\gamma_{HCO_3^-} = 1.01$$

Similarly,

$$\gamma_{Ca^{+2}} = 0.98$$

The saturation pH, pH_{sat}, at which $CaCO_3$ precipitation occurs is calculated from the following formula:

$$pH_{sat} = -\log\left(\frac{K_2\gamma_{Ca^{+2}}[Ca^{+2}]\gamma_{HCO_3^-}[HCO_3^-]}{K_{sp}}\right)$$

where $K_2 = 4.17\times10^{-11}$
and $K_{sp} = 5.25\times10^{-9}$

$$pH_{sat} = 13.7$$

The Langelier index is then,

$$LI = pH - pH_{sat} = -7.2$$

Water with a Langelier index <0 is corrosive (that is, undersaturated with respect to CaCO3). The Langelier index for our high quality deionised water is -7.2. This is an indication that it is highly corrosive.

C2.2 Christchurch Tap Water

Parameters (taken from CCC records from a typical Christchurch well):

TDS	107	mg/L			
$[Ca^{+2}]$	24.4	mg/L	$= 0.61\times10^{-3}$	mol/L	(40.0 g/mol)
$[HCO_3^-]$	57	mg/L	$= 0.94\times10^{-3}$	mol/L	(60.8 g/mol)
pH	7.9				

$\mu = 2.68\times10^{-3}$

$\gamma_{HCO_3^-} = 0.94$

$\gamma_{Ca^{+2}} = 0.80$

$pH_{sat} = 8.5$

$LI = pH - pH_{sat} = -0.6$

The Langelier index for Christchurch tap water shows it is also corrosive, but to a much less extent as the deionised water.